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Serum Calprotectin – A novel diagnostic and prognostic marker in Inflammatory Bowel Diseases

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Abstract

Introduction

There is an unmet need for novel blood based biomarkers that offer timely and accurate diagnostic and prognostic testing in Inflammatory Bowel Diseases (IBD). We aimed to investigate the diagnostic and prognostic utility of serum calprotectin (SC) in IBD.

Methods

A total of 171 patients (n=96 IBD, n=75 non-IBD) were prospectively recruited. A multi-biomarker model was derived using multivariable logistic regression analysis. Cox proportional hazards model was derived to assess the contribution of each variable to disease outcomes.

Results

SC correlated strongly with current biomarkers including faecal calprotectin (FC) (n=50, rho = 0.50, $p=1.6x10^{-4}$). SC was the strongest individual predictor of IBD diagnosis (odds ratio (OR): 9.37(95%CI: 2.82-34.68), $p=4.00\times10^{-4}$) compared with other markers (CRP: OR 8.52(95%CI: 2.75-28.63), $p=2.80\times10^{-4}$); albumin: OR 6.12(95%CI: 1.82-22.16), p=0.004). In a subset of 50 patients with paired SC and FC, the area under receiver operating characteristic discriminating IBD from controls was better for FC than SC (0.99, (95% CI 0.87-1.00) and 0.87 (95% CI:0.78-0.97) respectively; p=0.01).

At follow up (median 342 days; IQR: 88-563), SC predicted treatment escalation and/or surgery in IBD (HR 2.7, 95% CI: 1.1-4.9), in particular CD (HR 4.2, 95% CI 1.2-15.3).

A model incorporating SC and either CRP or albumin has a positive likelihood ratio of 24.14 for IBD. At 1 year, our prognostic model can predict treatment escalation in IBD in 65% of cases (95% CI: 43-79%) and 80% (95% CI: 31-94%) in CD if 2 or more blood marker criteria are met.

Conclusions

A diagnostic and prognostic model that combines SC and other blood-based biomarkers accurately predicts the inflammatory burden in IBD and has the potential to predict disease and its outcomes. Our data warrants further detailed exploration and validation in large multi-centre cohorts.

Introduction

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic, debilitating inflammatory disorders of the gastrointestinal tract affecting adults and children (1,2). A recent systematic review showed rising trends in the incidence and prevalence of IBD worldwide(3) associated with significant healthcare costs amounting to around £470 million in the UK, up to €5.6 billion annually in Europe and over $6 billion annually in the USA (3–8). With an ever expanding therapeutic repertoire, it is important to select patients who may benefit from early use of immunosuppressants' and/or biological therapies in order to minimise irreversible luminal damage and prevent long-term complications.

Blood-based biomarkers provide a non-invasive estimation of the inflammatory burden in newly diagnosed IBD. However relatively few blood-based biomarkers have been extensively validated in IBD, and fewer still are in routine use in the clinic (9). There is an emerging interest in discovering novel markers using multi-omic platforms that may be valuable in a variety of clinical settings including IBD diagnostics, disease activity assessments, predicting disease outcomes and response to therapy (9–11).

The S100 family of proteins including S100A8/A9 (calprotectin) and S100A12 (calgranulin) have been implicated in disease pathogenesis and investigated as potential markers of inflammation(12,13). In IBD, faecal calprotectin (FC) has emerged as a particularly informative tool(14). A recent meta-analysis of 13 studies and 1041 patients found that FC had a pooled sensitivity and specificity of 0.93 (0.85-0.97) and 0.96 (0.79-0.99) respectively for IBD and identified those individuals requiring endoscopy for suspected IBD(13). There are also data on the role of FC in other clinical settings, such as predicting post-operative CD recurrence and predicting outcomes in acute severe colitis(15,16). However, there are limitations to FC testing in clinical practice. Faecal collection can be a hurdle for patients(17)

and sample delivery and processing delays can hinder its clinical utility. In active UC, FC shows high within-day variability and the optimal timing for sampling is not clear(18,19). A blood based biomarker such as serum calprotectin (SC) may be more convenient in routine practice and more acceptable to patients. SC has been studied in diseases such as inflammatory arthropathies and cystic fibrosis (20–24). In Rheumatoid arthritis, SC was independently predictive of a 10 year radiographic disease progression (25), while in cystic fibrosis, SC predicted exacerbation and lung function decline(20,21).

More recently, SC has been investigated in IBD to predict response to- and relapse following anti-tumour necrosis factor (anti-TNF) therapy(17,26). In CD patients, SC has a similar profile to high sensitivity C-reactive protein (hsCRP) and compliments FC and hsCRP for prediction of relapse after anti-TNF withdrawal (p=0.0173, 0.0024 and 0.0002; HR: 3.191, 3.561 and 4.120 respectively) (17). In murine models, TNBS induced colitis is associated with higher SC levels that correlate closely to macroscopic and microscopic disease scores(27). We are yet to understand fully the relationship between SC and the other currently available biomarkers in IBD and the diagnostic and prognostic value of SC in IBD. Our study aims to investigate the role of SC in this clinical setting.

Methods

Study Design

A prospective, single centre case control study was performed in patients with suspected or confirmed IBD at their first presentation to a tertiary gastrointestinal clinic. Data were collected for patient demographics including age, sex, age at diagnosis and date of diagnosis (Table 1). Details of drug therapy and concomitant medications were recorded. Laboratory markers including C-reactive protein (CRP) and albumin were measured as part of the research protocol while other routine markers including haemoglobin, white cell count, platelets and faecal calprotectin were recorded within 30 days from recruitment.

Inclusion criteria

Patients with a new diagnosis of IBD were included in the study. The Lennard-Jones, Montreal and Paris criteria were used for diagnosis and classification of clinical phenotypes(28–30). The control cohort consisted of healthy lab volunteers (HC) and patients with gastrointestinal symptoms (symptomatic controls) who had no discernible inflammatory disease, and a diagnosis of functional bowel disease at follow up.

Sample collection and processing

For SC analysis, blood samples were collected prospectively and serum was processed within 2 hours of sampling (using centrifugation at 2500G for 15 min) and subsequently stored at −80°C until further use. Samples were analysed in duplicate using the Calpro™ AS calprotectin ELISA (Calpro AS, Norway) according to manufacturer's instructions. Samples with a calprotectin result of >2500 ng/ml were diluted and retested. Coefficients of variation of <10% were included in the analysis.

Ethics Statement

The NHS Lothian SAHSC Bioresource granted approval for this study (reference number SR558) with all patients giving written and informed consent (15/ES/0094).

Clinical Course in IBD

Case note review was performed for all IBD cases. Treatment escalation was defined as the need for escalation and establishment of 2 or more immunomodulatory therapies and/or surgery for disease flare after initial induction of disease remission (criteria previously used by Lee *et al*)(31). In UC, the definition of treatment escalation also included any patient with a new diagnosis, requiring emergency colectomy during their index admission.

Statistical analysis

Data were analysed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) and R 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Continuous data are

presented as medians and interquartile ranges and were analysed using a Mann–Whitney U-test. Categorical data are presented as numbers and percentages, and were analysed using Fisher's exact tests. Spearman's rank-order correlation test was applied for correlations between variables. To determine the accuracy of blood parameter measurements as a prognostic test capable of diagnosing IBD, receiver operating characteristic (ROC) analyses were performed by plotting sensitivity against specificity using the 'pROC' package in R(32).

Building diagnostic and prognostic models

After univariable analyses, the most significant laboratory parameters (CRP, albumin and serum calprotectin) were included in multivariable models for IBD diagnosis and prognosis. CRP and serum calprotectin were log transformed to more closely approximate a normal distribution for further multivariable analysis. ROC analyses were used to define the optimal cut-points (highest sum of sensitivity + specificity) for both models. The optimal models were then selected by performing backward stepwise regression using the smallest Akaike information criterion (AIC) values and adjusted for treatment exposure.

For the diagnostic model, an integer score was attributed to each variable according to its relative contribution in the model (as determined by the coefficients) and positive and negative predictive values were then calculated for each total score.

For the prognostic model, a Cox proportional hazards model was derived to assess the contribution of each variable to disease outcomes. Thresholds were then identified using ROC analyses to allow stratification of patients to either a benign or an aggressive disease course (requiring treatment escalation and/or surgery), and to allow creation of survival curves.

Results

Comparison of Serum Calprotectin with conventional biomarkers

Overall, serum calprotectin was analysed in 171 patient serum samples from August 2013 to April 2015. Serum calprotectin correlated positively with CRP (rho=0.61, $p=6.9x10^{-19}$) and negatively with albumin (rho= - 0.54, $p=3.3x10^{-14}$). Paired full blood count was available within 30 days (median 0 days; range: -26 to 16 days) of recruitment in 147 patients. Serum calprotectin correlated positively with Neutrophil count (rho=0.65, $p=1.9x10^{-20}$) and negatively with Haemoglobin (rho= - 0.42, $p=6.1 x10^{-8}$). Paired FC was available within 30 days (median 0 days, IQR: -5 to 5 days) of SC in 50 patients (IBD n=31, non-IBD n=19). Serum calprotectin correlated significantly with faecal calprotectin (Spearman's rho = 0.50, $p=1.6x10^{-4}$). Table 1 summarises the correlation of blood and faecal parameters. SC demonstrated a stronger correlation with white cell count (William's test, p=0.02) and neutrophils (p=0.03) in controls compared to IBD cases.

Diagnostic utility of Serum Calprotectin in IBD

In a subset of 156 patients (83 IBD and 73 non-IBD), blood sampling was performed within 90 days from diagnosis (median 0 days; IQR 0-6). There were 35 patients with a diagnosis of CD, 45 patients with UC and 3 patients with IBDU in the IBD group. In CD, 44% had L3 +/- L4 disease and 62% had B1 behaviour according to the Montreal classification (33). In UC, 33% had pancolitis (E4) and 11% had limited proctitis (E1) as per the Paris classification (30). Table 2 and Table 3 summarise the demographics and blood and faecal parameters for the IBD and control cohorts. SC was significantly increased in IBD compared with controls (1010 ng/ml [IQR 796-1426ng/ml] vs 506ng/ml [IQR 362-725ng/ml], $p=3.7 x 10^{-15}$) (Figure 1). CRP and albumin were also significantly different between IBD and controls (CRP $p=8.9x10^{-15}$; albumin $p=4.9x10^{-14}$). There was no difference in SC between CD and UC (1015 ng/ml [IQR 740-1518 ng/ml] vs 911µg/g [IQR 809-1413ng/ml], p=0.79) and within controls (HC: 432 ng/ml [IQR 359-586] vs symptomatic controls: 563ng/ml [IQR 382-787];

p=0.12). SC was not significantly associated with sex (p=0.14), age (rho -0.06, p=0.43) or smoking status (p=0.49). Serum calprotectin and CRP were able to discriminate IBD from controls with similar areas under the receiver operator characteristics curve (AUROC) of 0.87 (95% confidence interval [CI] 0.82-0.93) and 0.86 (95% CI 0.80-0.91) respectively (Figure 2) (p=0.64 DeLong's test for comparison of ROC curves). In those with paired SC and FC within 30 days, the AUROC for discriminating cases and controls was superior for FC (0.99, 95% CI 0.98-1.00) than SC (0.87, 95% CI 0.78-0.97) (p=0.01 De Long's test), as shown in Figure 2.

Multivariable analysis

Multivariable logistic regression analysis of predictors of IBD was performed on 155 cases (83 IBD, 72 non-IBD) where the data for the predictors were complete. Albumin, male gender, log transformed CRP and log transformed SC were significant predictors of IBD. **Table 4** summarises the statistical significance of each covariate.

Building an IBD Diagnosis Score

Using the multivariable model, continuous variables were categorised using integer cut-points guided by the ROC curves and observed relationship with diagnosis. The final scoring system for the diagnosis of IBD included SC>852ng/ml, Albumin<38g/L, CRP≥3.5mg/L and male gender. To formulate a numerical risk score, each variable was given a score based on the odds ratio generated from the linear model. **Table 5** summarises the positive and negative predictive values for each score. Using this model, a SC> 852ng/ml and either a CRP≥3.5mg/L or albumin <38g/L has a sensitivity of 67%, specificity of 97% and a positive likelihood ratio (LR) of 24.14 for IBD.

Predicting disease extent in IBD

SC, CRP and albumin were not able to differentiate between IBD subtypes (CRP p=0.45; albumin p=0.67; SC p=0.49). Within the UC cohort, SC was significantly higher in those

with disease beyond the rectum (> E1) compared to proctitis alone (E1) (median SC 1078ng/ml IQR 820-1418 vs 812ng/ml IQR 698-821, p=0.03). Albumin also predicted disease extent in UC (p=0.01) but not CRP (p=0.05). In CD however, there was no significant difference in SC, CRP or albumin by disease location (p=0.47, 0.55 and 0.20 respectively).

Predicting Disease outcomes in IBD

Kaplan-Meier analyses were performed on a total of 83 patients with IBD. There were 35 patients with a diagnosis of CD, 45 patients with UC and 3 patients with IBDU. The median age was 31 years (IQR: 26-41) and 69% were male (n=58). A total of 1(33%), 16 (46%), 23(51%) patients required treatment escalation in the IBDU, CD and UC group respectively. Using backwards stepwise selection, albumin<37 g/L and SC ≥1046 ng/ml remained significant predictors of treatment escalation in IBD (logrank test $p=5.1\times10^{-5}$). Both biomarkers had similar hazards ratio (HR) as shown in Table 6a. A score was generated using both biomarkers at these thresholds. At a year, the estimated chance of treatment escalation was 21% (95% CI: 1-37%) if none of the criteria were met, 40% (95% CI: 17-56%) for patients meeting one criterion and 65% (95% CI: 43-78%) for those meeting both criteria (**Figure 3a**).

In order to assess whether the time lag between diagnosis and blood sampling had an impact on the final model, stepwise regression analyses was performed for samples within 60 days (n=74) and 30 days (n=60) from diagnosis. SC remained a significant predictor of disease outcomes at 60 days and 30 days (p=0.003 and p=0.004 respectively).

In 28 patients, paired FC was available within 30 days from diagnosis. Using a multivariate model which included age, gender, CRP, albumin, FC and SC, backward stepwise regression analysis was performed and only SC remained as a significant predictor (p=0.0004). FC did not predict disease outcomes in this cohort (p=0.85, HR=1.0).

Further regression analyses were performed within the subgroups UC (**Table 6b**) and CD (**Table 6c**). In CD, CRP>24mg/L and SC>991ng/ml and albumin<26g/L predicted treatment escalation (logrank test p=0.003). At 1 year, the estimated chance of treatment escalation was 11% (95%CI: 0-29%) for patients meeting none of the criteria, 30% (95% CI: 0-51%) for patients meeting one criterion and 80% (95% CI: 31-94%) for patients meeting two or more criteria (**Figure 3b**).

In UC, albumin< 37g/L and CRP>2.5g/L predicted a more aggressive disease course (logrank test p=0.001). At 1 year, the estimated chance of treatment escalation was 0 for patients meeting none of the criteria, 38% (95% CI: 0-61%) for patients meeting one criterion and 68% (95% CI: 41-83%) for patients meeting two criteria (**Figure 3c**).

Discussion

There is an unmet need for accurate diagnostic and prognostic biomarkers in IBD as currently available blood biomarkers lack sensitivity and/or specificity. Our study is the first to investigate the role of SC in patients with a new diagnosis of IBD. SC independently predicts a diagnosis of IBD with an OR of 9.37 (95%CI: 2.82-34.68). A combined blood-based biomarker diagnostic model including SC and either CRP or albumin has a high positive LR for IBD (positive LR 24.14). Similarly, SC can predict treatment escalation and/or surgery in IBD (HR 2.7, 95%CI: 1.1-4.9), in particular CD (HR 4.2, 95% CI 1.2-15.3).

Calprotectin, a member of the S100 proteins, represents 45% of all cytosolic proteins in neutrophils compared to 1% in monocytes (34,35). Given the short half-life of SC (5 hours), it may provide a more dynamic test of the current inflammatory status compared with conventional inflammatory markers (half-life of CRP 18 hours, albumin 19 days) (36). SC correlates better with neutrophil count in controls compared to IBD patients; in IBD, SC levels may reflect calprotectin release from activated neutrophils and other immune cells such

as monocytes, macrophages and epithelial cells (**Figure 4**). SC shows a strong correlation with other markers such as CRP (r=0.61, $p=6.9x10^{-19}$) similar to published studies (r=0.33-0.59)(17,25,37,38) and a moderate correlation between SC and FC (0.50, $p=1.6x10^{-4}$).

As a diagnostic blood based marker, SC is the strongest predictor of IBD 9.37 (95%CI: 2.82-34.68). In a clinical setting, blood markers such as CRP are often available, therefore investigating the utility of a combined marker may be more relevant as this allows for greater specificity in diagnostics (39,40).We generated an IBD scoring system that would allow clinicians to predict IBD in patients at their index clinical visit. If 2 blood marker criteria are met (score of 8 or above), there is a high likelihood of IBD (positive LR 24.14).

FC has a high NPV but a low PPV for IBD vs functional disease (cut off 50 µg/g, NPV 93% PPV 37%)(41). In practice, a blood biomarker model can complement the existing FC screening of patients with gastrointestinal symptoms in primary care. In the current climate of optimal tertiary care resource management, this model can be utilised for patients being referred for suspected IBD and help select and prioritise investigations for individuals with a high IBD score and a high likelihood of disease. The AUC for FC is superior to SC in our study (0.87 and 0.99 respectively, p=0.01). FC has an established role in IBD diagnostics, however in clinical practice faecal sampling and testing can be challenging. One consideration in interpreting these data is the lag between SC and FC testing. The median time lag between SC and FC testing was 0 days (IQR -5 to 5 days), but there were individuals with upto 30 days between SC and FC testing. Nonetheless, any time lag represents real life experience with faecal testing as often FC is not available until a few weeks after the clinic visit. There is a large variability in the concentration of FC in stool within a single day and storage conditions can impact on FC levels(18). Sampling faeces can be a hurdle for patients and individuals can either decline FC testing, fail to provide a sample or provide insufficient sample for analysis. These factors impact on the practical utility of FC. SC testing has the

potential to provide a more timely assessment of inflammation on the day of the visit. The cost per sample for performing SC testing are comparable to FC (£5; $7.3 equivalent). In addition, other costs related to sample handling and processing are likely to be lower as serum testing is often automated.

Beyond diagnostics, studies have investigated the utility of non-invasive markers in predicting endoscopic activity. A recent meta-analyses evaluated the diagnostic accuracy of CRP, FC and stool lactoferrin (SL) for the assessment of endoscopically defined activity in IBD. The pooled AUC for CRP, FC and SL were 0.49(95% CI: 0.34-0.64), 0.88(CI: 0.84-0.90) and 0.73(CI: 0.66-0.79)(42). There was however heterogeneity in the endoscopic index used. Other factors such as inclusion criteria, in particular time lag between blood/faecal sampling and endoscopy (0-7 days) differed(43–46). There is a need for future prospective studies investigating the performance of non-invasive endoscopic activity markers such as SC.

In our study, SC predicts treatment escalation and/or surgery in IBD (HR 2.7, 95%CI: 1.1-4.9), in particular CD (HR 4.2, 95% CI 1.2-15.3). We also generate blood-based prognostic models incorporating CRP, albumin and SC. At 1 year, our model can predict treatment escalation in IBD in 65% of cases (95% CI: 43-79%) and 80% (95% CI: 31-94%) in CD if 2 or more criteria are met. Predicting the disease course early in individuals is becoming increasingly important in order to identify patients who would benefit from more aggressive therapy. In clinical practice, there is an unmet need for early indicators of persistent activity, either in a continuous or a relapsing-remitting manner despite initial induction therapy(31). These patients will often go on to require further immunomodulators, biological therapies and/or surgery. As quiescent IBD do not require such treatment escalations, we used the requirement of such treatment escalations to define an aggressive disease course (31). Clinical predictors have been studied previously. In CD, Beaugerie *et al* identified age, the

317 presence of perianal disease and requirement for steroids at diagnosis as independent
318 predictive factors for a disabling course(47). However, biological markers were not analysed
319 in that study. Since then, the role of biomarkers in predicting the disease course has been the
320 focus of many studies, although their effectiveness in predicting outcomes vary(31,48–51).
321 Most studies suggest that CRP predicts relapse in IBD(48–50), although one study found it
322 had no predictive value (52). There are several reasons for this observed variation and
323 includes differences in defining an aggressive disease course, disease heterogeneity and
324 disease duration prior to analyses. It is also possible that variations in CRP genotype may
325 explain variations in its performance in adult cohort studies. This has been described in the
326 paediatric population(53), but yet to be explored in adults. The role of FC in predicting
327 colectomy in acute severe colitis (ASUC) has been investigated previously (AUC 0.65,
328 p=0.04) and more recently, SC has been shown to predict colectomy in ASUC with an AUC
329 of 0.69 (95%CI 0.53-0.81) compared to FC (AUC 0.58; 95%CI 0.35-0.81) and CRP (AUC
330 0.71; 95%CI: 0.56-0.86) (16,38). SC has also been studied as a prognostic marker in
331 predicting relapse after anti-TNF withdrawal and complements FC (>250µg/g) and
332 hsCRP(>5mg/L) (p=0.0173, 0.0024 and 0.0002; HR: 3.191, 3.561 and 4.120 respectively)
333 (17). Our study however is the first to explore the prognostic utility of SC at diagnosis. Future
334 studies incorporating periodic SC testing to predict disease course in IBD may be useful.

335 Our study does have certain limitations. The results are from a single tertiary centre and
336 based on a select cohort of newly diagnosed IBD patients. The relatively small numbers
337 within the sub-type of IBD limits the power to dissect factors predicting phenotype and our
338 diagnostic and prognostic models require further validation. There were more females with
339 functional bowel disease in the control cohort and this alone may underly the observation that
340 male gender is a risk factor for IBD in our study. The major strengths of this study include a
341 prospective design and a cohort of newly diagnosed IBD aiming for the first time to explore

the correlation of SC with current biomarkers and build diagnostic and prognostic models for potential clinical use in IBD.

Conclusion

SC shows promise as a blood based biomarker in diagnosing and predicting disease course in IBD. A diagnostic and prognostic model that combines SC and other blood-based biomarkers accurately predicts the inflammatory burden in IBD and has the potential to predict disease and its outcomes. Our findings warrant further exploration and validation within large multicentre cohorts.

What is Current Knowledge

- Serum calprotectin (SC) has been studied as a prognostic marker in predicting relapse after anti-TNF withdrawal and complements FC and hsCRP for the prediction of relapse.
- SC can predict colectomy in acute severe colitis with an AUC of 0.69, comparable to CRP.
- SC correlates with other inflammatory blood markers such as CRP
- SC has been studied in diseases such as inflammatory arthropathies and cystic fibrosis and can predict disease progression.

What is New Here

- Serum Calprotectin (SC) is a strong individual predictor of a diagnosis of IBD
- SC correlates with faecal calprotectin (FC) and is useful in diagnosis
- SC can predict treatment escalation and/or surgery in IBD, in particular CD
- Blood based diagnostic and prognostic models can provide an accurate reflection of the inflammatory burden and have the potential to predict disease and its outcomes.

Competing interests

The study was supported by Calpro AS™, Norway who provided the ELISA kits for serum calprotectin testing.

Author contributions

Study design RK and JS. Patient recruitment and sample processing NTV, RK, NAK, RKB. Experimental work RK and MV. Data Analysis RK, NAK, ATA and NTV. RK wrote the manuscript. All authors were involved in critical review, editing, revision and approval of the final manuscript.

References

1. Kalla R, Ventham NT, Satsangi J, *et al.* Crohn's disease. BMJ 2014;349:g6670.
2. Ford AC, Moayyedi P, Hanauer SB. Ulcerative colitis. BMJ 2013;346:f432.
3. Molodecky NA, Soon IS, Rabi DM, *et al.* Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 2012;142:46–54.e42; quiz e30.
4. IBD standards group.Quality care: Service standards for the healthcare of people who have inflammatory bowel disease (IBD). 2009.
5. Loftus E V. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. Gastroenterology 2004;126:1504–1517.
6. Kappelman MD, Rifas-Shiman SL, Kleinman K, *et al.* The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. Clin. Gastroenterol. Hepatol. 2007;5:1424–9.
7. Kappelman MD, Rifas-Shiman SL, Porter CQ, *et al.* Direct Health Care Costs of Crohn's Disease and Ulcerative Colitis in US Children and Adults. Gastroenterology 2008;135:1907–1913.
8. Burisch J, Jess T, Martinato M, *et al.* The burden of inflammatory bowel disease in Europe. J. Crohns. Colitis 2013;7:322–37.
9. Sands BE. Biomarkers of Inflammation in Inflammatory Bowel Disease. Gastroenterology 2015;
10. Viennois E, Zhao Y, Merlin D. Biomarkers of Inflammatory Bowel Disease: From Classical Laboratory Tools to Personalized Medicine. Inflamm. Bowel Dis. 2015;21:2467–74.

11. P140. Proximity Extension Assay technology identifies novel serum biomarkers for predicting Inflammatory Bowel Disease: IBD Character Consortium. J. Crohns. Colitis 2015;9 Suppl 1:S146–7.

12. Kaiser T, Langhorst J, Wittkowski H, *et al.* Faecal S100A12 as a non-invasive marker distinguishing inflammatory bowel disease from irritable bowel syndrome. Gut 2007;56:1706–13.

13. Rheenen PF van, Vijver E Van de, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. BMJ 2010;341:c3369.

14. Lehmann FS, Burri E, Beglinger C. The role and utility of faecal markers in inflammatory bowel disease. Therap. Adv. Gastroenterol. 2015;8:23–36.

15. Wright EK, Kamm MA, Cruz P De, *et al.* Measurement of Fecal Calprotectin Improves Monitoring and Detection of Recurrence of Crohn's Disease Following Surgery. Gastroenterology 2015;

16. Ho GT, Lee HM, Brydon G, *et al.* Fecal calprotectin predicts the clinical course of acute severe ulcerative colitis. Am. J. Gastroenterol. 2009;104:673–678.

17. Meuwis M-A, Vernier-Massouille G, Grimaud JC, *et al.* Serum calprotectin as a biomarker for Crohn's disease. J. Crohns. Colitis 2013;7:e678–83.

18. Lasson A, Stotzer P-O, Ohman L, *et al.* The intra-individual variability of faecal calprotectin: A prospective study in patients with active ulcerative colitis. J. Crohns. Colitis 2014;

19. Calafat M, Cabré E, Mañosa M, *et al.* High within-day variability of fecal calprotectin levels in patients with active ulcerative colitis: what is the best timing for stool sampling? Inflamm. Bowel Dis. 2015;21:1072–6.

20. Reid PA, McAllister DA, Boyd AC, *et al.* Measurement of serum calprotectin in stable patients predicts exacerbation and lung function decline in cystic fibrosis. Am. J. Respir. Crit. Care Med. 2015;191:233–6.

21. Gray RD, Imrie M, Boyd AC, *et al.* Sputum and serum calprotectin are useful biomarkers during CF exacerbation. J. Cyst. Fibros. 2010;9:193–8.

22. Abildtrup M, Kingsley GH, Scott DL. Calprotectin as a Biomarker for Rheumatoid Arthritis: A Systematic Review. J. Rheumatol. 2015;

23. Obry A, Lequerré T, Hardouin J, *et al.* Identification of S100A9 as biomarker of responsiveness to the methotrexate/etanercept combination in rheumatoid arthritis using a proteomic approach. PLoS One 2014;9:e115800.

24. Mariani A, Marsili M, Nozzi M, *et al.* Serum calprotectin: review of its usefulness and validity in paediatric rheumatic diseases. Clin. Exp. Rheumatol. 2014;33:109–114.

25. Hammer HB, Ødegård S, Syversen SW, *et al.* Calprotectin (a major S100 leucocyte protein) predicts 10-year radiographic progression in patients with rheumatoid arthritis. Ann. Rheum. Dis. 2010;69:150–4.

26. Boschetti G, Garnero P, Moussata D, *et al.* Accuracies of Serum and Fecal S100 Proteins (Calprotectin and Calgranulin C) to Predict the Response to TNF Antagonists in Patients with Crohn's Disease. Inflamm. Bowel Dis. 2015;21:331–6.

27. Cury DB, Mizsputen SJ, Versolato C, *et al.* Serum calprotectin levels correlate with biochemical and histological markers of disease activity in TNBS colitis. Cell. Immunol. 2013;282:66–70.

28. Silverberg MS, Satsangi J, Ahmad T, *et al.* Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can. J.

458 Gastroenterol. 2005;19 Suppl A:5A–36A.

459 29. Lennard-Jones JE. Classification of inflammatory bowel disease. Scand. J.
460 Gastroenterol. Suppl. 1989;170:2–6; discussion 16–9.

461 30. Levine A, Griffiths A, Markowitz J, *et al.* Pediatric modification of the Montreal
462 classification for inflammatory bowel disease: the Paris classification. Inflamm. Bowel
463 Dis. 2011;17:1314–21.

464 31. Lee JC, Lyons P a, McKinney EF, *et al.* Gene expression profiling of CD8+ T cells
465 predicts prognosis in patients with Crohn disease and ulcerative colitis. J. Clin. Invest.
466 2011;121:4170–9.

467 32. Robin X, Turck N, Hainard A, *et al.* pROC: an open-source package for R and S+ to
468 analyze and compare ROC curves. BMC Bioinformatics 2011;12:77.

469 33. Satsangi J, Silverberg MS, Vermeire S, *et al.* The Montreal classification of
470 inflammatory bowel disease: controversies, consensus, and implications. Gut
471 2006;55:749–53.

472 34. Averill MM, Kerkhoff C, Bornfeldt KE. S100A8 and S100A9 in cardiovascular
473 biology and disease. Arterioscler. Thromb. Vasc. Biol. 2012;32:223–9.

474 35. Ingersoll MA, Spanbroek R, Lottaz C, *et al.* Comparison of gene expression profiles
475 between human and mouse monocyte subsets. Blood 2010;115:e10–9.

476 36. Fagerhol MK, Nielsen HG, Vetlesen A, *et al.* Increase in plasma calprotectin during
477 long-distance running. 2005.

478 37. Leach ST, Yang Z, Messina I, *et al.* Serum and mucosal S100 proteins, calprotectin
479 (S100A8/S100A9) and S100A12, are elevated at diagnosis in children with
480 inflammatory bowel disease. Scand. J. Gastroenterol. 2007;42:1321–31.

38. Hare NC, Kennedy NA, Kalla R, *et al.* P349 Serum calprotectin: a novel biomarker to predict outcome in acute severe ulcerative colitis? J. Crohn's Colitis 2014;8:S210.

39. Mor G, Visintin I, Lai Y, *et al.* Serum protein markers for early detection of ovarian cancer. Proc. Natl. Acad. Sci. U. S. A. 2005;102:7677–82.

40. Xiao T, Ying W, Li L, *et al.* An approach to studying lung cancer-related proteins in human blood. Mol. Cell. Proteomics 2005;4:1480–6.

41. Kennedy NA, Clark A, Walkden A, *et al.* Clinical utility and diagnostic accuracy of faecal calprotectin for IBD at first presentation to gastroenterology services in adults aged 16-50 years. J. Crohns. Colitis 2015;9:41–9.

42. Mosli MH, Zou G, Garg SK, *et al.* C-Reactive Protein, Fecal Calprotectin, and Stool Lactoferrin for Detection of Endoscopic Activity in Symptomatic Inflammatory Bowel Disease Patients: A Systematic Review and Meta-Analysis. Am. J. Gastroenterol. 2015;110:802–819.

43. af Björkesten C-G, Nieminen U, Turunen U, *et al.* Surrogate markers and clinical indices, alone or combined, as indicators for endoscopic remission in anti-TNF-treated luminal Crohn's disease. Scand. J. Gastroenterol. 2012;47:528–37.

44. D'Haens G, Ferrante M, Vermeire S, *et al.* Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm. Bowel Dis. 2012;18:2218–24.

45. Schoepfer AM, Beglinger C, Straumann A, *et al.* Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. Am. J. Gastroenterol. 2010;105:162–9.

46. Langhorst J, Elsenbruch S, Koelzer J, *et al.* Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal

lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. Am. J. Gastroenterol. 2008;103:162–9.

47. Beaugerie L, Seksik P, Nion-Larmurier I, *et al.* Predictors of crohn's disease. Gastroenterology 2006;130:650–656.

48. Boirivant M, Leoni M, Tariciotti D, *et al.* The clinical significance of serum C reactive protein levels in Crohn's disease. Results of a prospective longitudinal study. J. Clin. Gastroenterol. 1988;10:401–405.

49. Niewiadomski O, Studd C, Hair C, *et al.* Prospective population-based cohort of inflammatory bowel disease in the biologics era: Disease course and predictors of severity. J. Gastroenterol. Hepatol. 2015;30:1346–53.

50. Henriksen M, Jahnsen J, Lygren I, *et al.* C-reactive protein: a predictive factor and marker of inflammation in inflammatory bowel disease. Results from a prospective population-based study. Gut 2008;57:1518–1523.

51. Sandor Kiss L, Papp M, Dorottya Lovasz B, *et al.* High-sensitivity C-reactive protein for identification of disease phenotype, active disease, and clinical relapses in Crohn's disease: A marker for patient classification? Inflamm. Bowel Dis. 2012;18:1647–1654.

52. Costa F, Mumolo MG, Ceccarelli L, *et al.* Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. Gut 2005;54:364–368.

53. Henderson P, Kennedy NA, Limbergen JE Van, *et al.* Serum C-reactive protein and CRP genotype in pediatric inflammatory bowel disease: influence on phenotype, natural history, and response to therapy. Inflamm. Bowel Dis. 2015;21:596–605.

Figure Legends

Figure 1: Serum Calprotectin levels in patients with Crohn's disease (CD), Ulcerative colitis (UC), Inflammatory bowel disease unclassified (IBDU), symptomatic controls (non-IBD) and healthy controls (HC)

Footnote: Boxplots represent median and inter-quartile ranges for serum calprotectin within each subcohort

Figure 2: Receiver operating curve analysis (ROC) of serum calprotectin (SC) and other blood based markers in differentiating Inflammatory bowel diseases (IBD) from non-IBD and ROC analysis of SC and faecal calprotectin (FC) (within 30 days) in discriminating Inflammatory bowel diseases (IBD) from non-IBD.

Footnote: WCC:white cell count; CRP:C-reactive protein; Hb:Haemoglobin

Figure 3a: Kaplan Meier survival curves of disease course using blood biomarkers to predict outcomes in newly diagnosed Inflammatory Bowel Diseases (IBD). Single marker represents either or albumin<37 g/L or serum calprotectin ≥ 1046ng/ml. Dual markers represents a combination of both variables.

Figure 3b: Kaplan Meier survival curves of disease course using blood biomarkers to predict outcomes in newly diagnosed Crohn's disease (CD)

Footnote: '1 marker' represents either CRP>24mg/L or albumin<26 g/L or serum calprotectin >991 ng/ml. '2 or 3 marker' represents a combination of any 2 or 3 of the above mentioned variables.

549 **Figure 3c**: Kaplan Meier survival curves of disease course using blood biomarkers to predict
550 outcomes in newly diagnosed Ulcerative Colitis (UC)

551 **Footnote**: Single marker represents either albumin<37 g/L or CRP >2.5mg/L. Dual markers
552 represents all the categorical variables as a combined biomarker.

553 **Figure 4**: Correlation between log transformed serum calprotectin and neutrophil count in
554 Inflammatory Bowel Diseases (IBD) versus controls (Non-IBD).

Serum Calprotectin – A novel diagnostic and prognostic marker in Inflammatory Bowel Diseases

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Abstract

Introduction

There is an unmet need for novel blood based biomarkers that offer timely and accurate diagnostic and prognostic testing in Inflammatory Bowel Diseases (IBD). We aimed to investigate the diagnostic and prognostic utility of serum calprotectin (SC) in IBD.

Methods

A total of 171 patients (n=96 IBD, n=75 non-IBD) were prospectively recruited. A multi-biomarker model was derived using multivariable logistic regression analysis. Cox proportional hazards model was derived to assess the contribution of each variable to disease outcomes.

Results

SC correlated strongly with current biomarkers including faecal calprotectin (FC) (n=50, rho = 0.50, $p=1.6x10^{-4}$). SC was the strongest individual predictor of IBD diagnosis (odds ratio (OR): 9.37(2.82-34.68), $p=4.00\times10^{-4}$) compared with other markers (CRP: OR 8.52(95%CI: 2.75-28.63), $p=2.80\times10^{-4}$); albumin: OR 6.12(95%CI: 1.82-22.16), p=0.004). In a subset of 50 patients with paired SC and FC, the area under receiver operating characteristic discriminating IBD from controls was better for FC than SC (0.99, (95% CI 0.87-1.00) and 0.87 (95% CI: 0.78-0.97) respectively; p=0.01).

At follow up (median 342 days; IQR: 88-563), **SC predicted treatment escalation and/or surgery in IBD (HR 2.7, 95% CI: 1.1-4.9), in particular CD (HR 4.2, 95% CI 1.2-15.3).**

A model incorporating SC and either CRP or albumin has a positive likelihood ratio of 24.14 for IBD. At 1 year, our prognostic model can predict treatment escalation in IBD in 65% of cases (95% CI: 43-79%) and 80% (95% CI: 31-94%) in CD if 2 or more blood marker criteria are met.

Conclusions

A diagnostic and prognostic model that combines SC and other blood-based biomarkers accurately predicts the inflammatory burden in IBD and has the potential to predict disease and its outcomes. Our data warrants further detailed exploration and validation in large multi-centre cohorts.

Introduction

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic, debilitating inflammatory disorders of the gastrointestinal tract affecting adults and children (1,2). A recent systematic review showed rising trends in the incidence and prevalence of IBD worldwide(3) associated with significant healthcare costs amounting to around £470 million in the UK, up to €5.6 billion annually in Europe and over $6 billion annually in the USA (3–8). With an ever expanding therapeutic repertoire, it is important to select patients who may benefit from early use of immunosuppressants' and/or biological therapies in order to minimise irreversible luminal damage and prevent long-term complications.

Blood-based biomarkers provide a non-invasive estimation of the inflammatory burden in newly diagnosed IBD. However relatively few blood-based biomarkers have been extensively validated in IBD, and fewer still are in routine use in the clinic (9). There is an emerging interest in discovering novel markers using multi-omic platforms that may be valuable in a variety of clinical settings including IBD diagnostics, disease activity assessments, predicting disease outcomes and response to therapy (9–11).

The S100 family of proteins including S100A8/A9 (calprotectin) and S100A12 (calgranulin) have been implicated in disease pathogenesis and investigated as potential markers of inflammation(12,13). In IBD, faecal calprotectin (FC) has emerged as a particularly informative tool(14). A recent meta-analysis of 13 studies and 1041 patients found that FC had a pooled sensitivity and specificity of 0.93 (0.85-0.97) and 0.96 (0.79-0.99) respectively for IBD and identified those individuals requiring endoscopy for suspected IBD(13). There are also data on the role of FC in other clinical settings, such as predicting post-operative CD recurrence and predicting outcomes in acute severe colitis(15,16). However, there are limitations to FC testing in clinical practice. Faecal collection can be a hurdle for patients(17)

and sample delivery and processing delays can hinder its clinical utility. In active UC, FC shows high within-day variability and the optimal timing for sampling is not clear(18,19). A blood based biomarker such as serum calprotectin (SC) may be more convenient in routine practice and more acceptable to patients. SC has been studied in diseases such as inflammatory arthropathies and cystic fibrosis (20–24). In Rheumatoid arthritis, SC was independently predictive of a 10 year radiographic disease progression (25), while in cystic fibrosis, SC predicted exacerbation and lung function decline(20,21).

More recently, SC has been investigated in IBD to predict response to- and relapse following anti-tumour necrosis factor (anti-TNF) therapy(17,26). In CD patients, SC has a similar profile to high sensitivity C-reactive protein (hsCRP) and compliments FC and hsCRP for prediction of relapse after anti-TNF withdrawal (p=0.0173, 0.0024 and 0.0002; HR: 3.191, 3.561 and 4.120 respectively) (17). In murine models, TNBS induced colitis is associated with higher SC levels that correlate closely to macroscopic and microscopic disease scores(27). We are yet to understand fully the relationship between SC and the other currently available biomarkers in IBD and the diagnostic and prognostic value of SC in IBD. Our study aims to investigate the role of SC in this clinical setting.

Methods

Study Design

A prospective, single centre case control study was performed in patients with suspected or confirmed IBD at their first presentation to a tertiary gastrointestinal clinic. Data were collected for patient demographics including age, sex, age at diagnosis and date of diagnosis (**Table 1**). Details of drug therapy and concomitant medications were recorded. Laboratory **markers including C-reactive protein (CRP) and albumin were measured as part of the research protocol while other routine markers including haemoglobin, white cell count, platelets and faecal calprotectin were recorded within 30 days from recruitment.**

Inclusion criteria

Patients with a new diagnosis of IBD were included in the study. The Lennard-Jones, Montreal and Paris criteria were used for diagnosis and classification of clinical phenotypes(28–30). **The control cohort consisted of healthy lab volunteers (HC) and patients with gastrointestinal symptoms (symptomatic controls) who had no discernible inflammatory disease, and a diagnosis of functional bowel disease at follow up.**

Sample collection and processing

For SC analysis, blood samples were collected prospectively and serum was processed within 2 hours of sampling (using centrifugation at 2500G for 15 min) and subsequently stored at −80°C until further use. Samples were analysed in duplicate using the Calpro™ AS calprotectin ELISA (Calpro AS, Norway) according to manufacturer's instructions. Samples with a calprotectin result of >2500 ng/ml were diluted and retested. Coefficients of variation of <10% were included in the analysis.

Ethics Statement

The NHS Lothian SAHSC Bioresource granted approval for this study (reference number SR558) with all patients giving written and informed consent (15/ES/0094).

Clinical Course in IBD

Case note review was performed for all IBD cases. Treatment escalation was defined as the need for escalation and establishment of 2 or more immunomodulatory therapies and/or surgery for disease flare after initial induction of disease remission (criteria previously used by Lee *et al*)(31). In UC, the definition of treatment escalation also included any patient with a new diagnosis, requiring emergency colectomy during their index admission.

Statistical analysis

Data were analysed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) and R 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Continuous data are

presented as medians and interquartile ranges and were analysed using a Mann–Whitney U-test. Categorical data are presented as numbers and percentages, and were analysed using Fisher's exact tests. Spearman's rank-order correlation test was applied for correlations between variables. To determine the accuracy of blood parameter measurements as a prognostic test capable of diagnosing IBD, receiver operating characteristic (ROC) analyses were performed by plotting sensitivity against specificity using the 'pROC' package in R(32).

Building diagnostic and prognostic models

After univariable analyses, the most significant laboratory parameters (CRP, albumin and serum calprotectin) were included in multivariable models for IBD diagnosis and prognosis. CRP and serum calprotectin were log transformed to more closely approximate a normal distribution for further multivariable analysis. ROC analyses were used to define the optimal cut-points (highest sum of sensitivity + specificity) for both models. The optimal models were then selected by performing backward stepwise regression using the smallest Akaike information criterion (AIC) values **and adjusted for treatment exposure**.

For the diagnostic model, an integer score was attributed to each variable according to its relative contribution in the model (as determined by the coefficients) and positive and negative predictive values were then calculated for each total score.

For the prognostic model, a Cox proportional hazards model was derived to assess the contribution of each variable to disease outcomes. Thresholds were then identified using ROC analyses to allow stratification of patients to either a benign or an aggressive disease course (requiring treatment escalation and/or surgery), and to allow creation of survival curves.

Results

Comparison of Serum Calprotectin with conventional biomarkers

Overall, serum calprotectin was analysed in 171 patient serum samples from August 2013 to April 2015. **Serum calprotectin correlated positively with CRP (rho=0.61, $p=6.9x10^{-19}$) and negatively with albumin (rho= - 0.54, $p=3.3x10^{-14}$). Paired full blood count was available within 30 days (median 0 days; range: -26 to 16 days) of recruitment in 147 patients. Serum calprotectin correlated positively with Neutrophil count (rho=0.65, $p=1.9x10^{-20}$) and negatively with Haemoglobin (rho= - 0.42, $p=6.1 x10^{-8}$). Paired FC was available within 30 days (median 0 days, IQR: -5 to 5 days) of SC in 50 patients (IBD n=31, non-IBD n=19). Serum calprotectin correlated significantly with faecal calprotectin (Spearman's rho = 0.50, $p=1.6x10^{-4}$).** **Table 1** summarises the correlation of blood and faecal parameters. SC demonstrated a stronger correlation with white cell count (William's test, p=0.02) and neutrophils (p=0.03) in controls compared to IBD cases.

Diagnostic utility of Serum Calprotectin in IBD

In a subset of 156 patients (83 IBD and 73 non-IBD), blood sampling was performed within 90 days from diagnosis (median 0 days; IQR 0-6). There were 35 patients with a diagnosis of CD, 45 patients with UC and 3 patients with IBDU in the IBD group. In CD, 44% had L3 +/- L4 disease and 62% had B1 behaviour according to the Montreal classification (33). In UC, 33% had pancolitis (E4) and 11% had limited proctitis (E1) as per the Paris classification (30). **Table 2** and **Table 3** summarise the demographics and blood and faecal parameters for the IBD and control cohorts. SC was significantly increased in IBD compared with controls **(1010 ng/ml [IQR 796-1426ng/ml] vs 506ng/ml [IQR 362-725ng/ml], $p=3.7 x 10^{-15}$)** (**Figure 1**). CRP and albumin were also significantly different between IBD and controls **(CRP $p=8.9x10^{-15}$; albumin $p=4.9x10^{-14}$).** There was no difference in SC between CD and UC **(1015 ng/ml [IQR 740-1518 ng/ml] vs 911µg/g [IQR 809-1413ng/ml], p=0.79) and within controls (HC: 432 ng/ml [IQR 359-586] vs**

symptomatic controls: 563ng/ml [IQR 382-787]; p=0.12). SC was not significantly associated with sex (p=0.14), age (rho -0.06, p=0.43) or smoking status (p=0.49). Serum calprotectin and CRP were able to discriminate IBD from controls with similar areas under **the receiver operator characteristics curve (AUROC) of 0.87 (95% confidence interval [CI] 0.82-0.93) and 0.86 (95% CI 0.80-0.91) respectively (Figure 2) (p=0.64 DeLong's test for comparison of ROC curves). In those with paired SC and FC within 30 days, the AUROC for discriminating cases and controls was superior for FC (0.99, 95% CI 0.98-1.00) than SC (0.87, 95% CI 0.78-0.97) (p=0.01 De Long's test), as shown in Figure 2.**

Multivariable analysis

Multivariable logistic regression analysis of predictors of IBD was performed on 155 cases (83 IBD, 72 non-IBD) where the data for the predictors were complete. Albumin, male gender, log transformed CRP and log transformed SC were significant predictors of IBD. **Table 4** summarises the statistical significance of each covariate.

Building an IBD Diagnosis Score

Using the multivariable model, continuous variables were categorised using integer cut-points guided by the ROC curves and observed relationship with diagnosis. The final scoring system for the diagnosis of IBD included SC>852ng/ml, Albumin<38g/L, CRP≥3.5mg/L and male gender. To formulate a numerical risk score, each variable was given a score based on the odds ratio generated from the linear model. **Table 5** summarises the positive and negative predictive values for each score. **Using this model, a SC> 852ng/ml and either a CRP≥3.5mg/L or albumin <38g/L has a sensitivity of 67%, specificity of 97% and a positive likelihood ratio (LR) of 24.14 for IBD.**

Predicting disease extent in IBD

SC, CRP and albumin were not able to differentiate between IBD subtypes (CRP p=0.45; albumin p=0.67; SC p=0.49). Within the UC cohort, SC was significantly higher in those

with disease beyond the rectum (> E1) compared to proctitis alone (E1) (median SC 1078ng/ml IQR 820-1418 vs 812ng/ml IQR 698-821, p=0.03). Albumin also predicted disease extent in UC (p=0.01) but not CRP (p=0.05). In CD however, there was no significant difference in SC, CRP or albumin by disease location (p=0.47, 0.55 and 0.20 respectively).

Predicting Disease outcomes in IBD

Kaplan-Meier analyses were performed on a total of 83 patients with IBD. There were 35 patients with a diagnosis of CD, 45 patients with UC and 3 patients with IBDU. The median age was 31 years (IQR: 26-41) and 69% were male (n=58). A total of 1(33%), 16 (46%), 23(51%) patients required treatment escalation in the IBDU, CD and UC group respectively. **Using backwards stepwise selection, albumin<37 g/L and SC ≥1046 ng/ml remained significant predictors of treatment escalation in IBD (logrank test $p=5.1\times10^{-5}$). Both biomarkers had similar hazards ratio (HR) as shown in Table 6a. A score was generated using both biomarkers at these thresholds. At a year, the estimated chance of treatment escalation was 21% (95% CI: 1-37%) if none of the criteria were met, 40% (95% CI: 17-56%) for patients meeting one criterion and 65% (95% CI: 43-78%) for those meeting both criteria** (**Figure 3a**).

In order to assess whether the time lag between diagnosis and blood sampling had an impact on the final model, stepwise regression analyses was performed for samples within 60 days (n=74) and 30 days (n=60) from diagnosis. SC remained a significant predictor of disease outcomes at 60 days and 30 days (p=0.003 and p=0.004 respectively).

In 28 patients, paired FC was available within 30 days from diagnosis. Using a multivariate model which included age, gender, CRP, albumin, FC and SC, backward stepwise regression analysis was performed and only SC remained as a significant predictor (p=0.0004). FC did not predict disease outcomes in this cohort (p=0.85, HR=1.0).

Further regression analyses were performed within the subgroups UC (**Table 6b**) and CD (**Table 6c**). In CD, CRP>24mg/L and SC>991ng/ml and albumin<26g/L predicted treatment escalation (logrank test p=0.003). At 1 year, the estimated chance of treatment escalation was 11% (95%CI: 0-29%) for patients meeting none of the criteria, 30% (95% CI: 0-51%) for patients meeting one criterion and 80% (95% CI: 31-94%) for patients meeting two or more criteria (**Figure 3b**).

In UC, albumin< 37g/L and CRP>2.5g/L predicted a more aggressive disease course (logrank test p=0.001). At 1 year, the estimated chance of treatment escalation was 0 for patients meeting none of the criteria, 38% (95% CI: 0-61%) for patients meeting one criterion and 68% (95% CI: 41-83%) for patients meeting two criteria (**Figure 3c**).

Discussion

There is an unmet need for accurate diagnostic and prognostic biomarkers in IBD as currently available blood biomarkers lack sensitivity and/or specificity. Our study is the first to investigate the role of SC in patients with a new diagnosis of IBD. **SC independently predicts a diagnosis of IBD with an OR of 9.37 (95%CI: 2.82-34.68). A combined blood-based biomarker diagnostic model including SC and either CRP or albumin has a high positive LR for IBD (positive LR 24.14). Similarly, SC can predict treatment escalation and/or surgery in IBD (HR 2.7, 95%CI: 1.1-4.9), in particular CD (HR 4.2, 95% CI 1.2-15.3).**

Calprotectin, a member of the S100 proteins, represents 45% of all cytosolic proteins in neutrophils compared to 1% in monocytes (34,35). Given the short half-life of SC (5 hours), it may provide a more dynamic test of the current inflammatory status compared with conventional inflammatory markers (half-life of CRP 18 hours, albumin 19 days) (36). SC correlates better with neutrophil count in controls compared to IBD patients; in IBD, SC

levels may reflect calprotectin release from activated neutrophils and other immune cells such as monocytes, macrophages and epithelial cells (**Figure 4**). SC shows a strong correlation with other markers such as CRP (r=0.61, $p=6.9x10^{-19}$) similar to published studies (r=0.33-0.59)(17,25,37,38) and **a moderate correlation between SC and FC (0.50, $p=1.6x10^{-4}$).**

As a diagnostic blood based marker, SC is the strongest predictor of IBD 9.37 (95%CI: 2.82-34.68). In a clinical setting, blood markers such as CRP are often available, therefore investigating the utility of a combined marker may be more relevant as this allows for greater specificity in diagnostics (39,40).We generated an IBD scoring system that would allow clinicians to predict IBD in patients at their index clinical visit. **If 2 blood marker criteria are met (score of 8 or above), there is a high likelihood of IBD (positive LR 24.14).**

FC has a high NPV but a low PPV for IBD vs functional disease (cut off 50 μg/g, NPV 93% PPV 37%)(41). In practice, a blood biomarker model can complement the existing FC screening of patients with gastrointestinal symptoms in primary care. In the current climate of optimal tertiary care resource management, this model can be utilised for patients being referred for suspected IBD and help select and prioritise investigations for individuals with a high IBD score and a high likelihood of disease. **The AUC for FC is superior to SC in our study (0.87 and 0.99 respectively, p=0.01).** FC has an established role in IBD diagnostics, however in clinical practice faecal sampling and testing can be challenging. One consideration in interpreting these data is the lag between SC and FC testing. The median time lag between SC and FC testing was 0 days (IQR -5 to 5 days), but there were individuals with upto 30 days between SC and FC testing. Nonetheless, any time lag represents real life experience with faecal testing as often FC is not available until a few weeks after the clinic visit. There is a large variability in the concentration of FC in stool within a single day and storage conditions can impact on FC levels(18). **Sampling faeces can be a hurdle for patients and individuals can either decline FC testing, fail to provide a sample or**

provide insufficient sample for analysis. These factors impact on the practical utility of FC. SC testing has the potential to provide a more timely assessment of inflammation on the day of the visit. **The cost per sample for performing SC testing are comparable to FC (£5; $7.3 equivalent). In addition, other costs related to sample handling and processing are likely to be lower as serum testing is often automated.**

Beyond diagnostics, studies have investigated the utility of non-invasive markers in predicting endoscopic activity. A recent meta-analyses evaluated the diagnostic accuracy of CRP, FC and stool lactoferrin (SL) for the assessment of endoscopically defined activity in IBD. The pooled AUC for CRP, FC and SL were 0.49(95% CI: 0.34-0.64), 0.88(CI: 0.84-0.90) and 0.73(CI: 0.66-0.79)(42). There was however heterogeneity in the endoscopic index used. Other factors such as inclusion criteria, in particular time lag between blood/faecal sampling and endoscopy (0-7 days) differed(43–46). There is a need for future prospective studies investigating the performance of non-invasive endoscopic activity markers such as SC.

In our study, SC predicts treatment escalation and/or surgery in IBD (HR 2.7, 95%CI: 1.1-4.9), in particular CD (HR 4.2, 95% CI 1.2-15.3). We also generate blood-based prognostic models incorporating CRP, albumin and SC. At 1 year, our model can predict treatment escalation in IBD in 65% of cases (95% CI: 43-79%) and 80% (95% CI: 31-94%) in CD if 2 or more criteria are met. Predicting the disease course early in individuals is becoming increasingly important in order to identify patients who would benefit from more aggressive therapy. In clinical practice, there is an unmet need for early indicators of persistent activity, either in a continuous or a relapsing-remitting manner despite initial induction therapy(31). These patients will often go on to require further immunomodulators, biological therapies and/or surgery. As quiescent IBD do not require such treatment escalations, we used the requirement of such treatment escalations to define an aggressive

disease course (31). Clinical predictors have been studied previously. In CD, Beaugerie *et al* identified age, the presence of perianal disease and requirement for steroids at diagnosis as independent predictive factors for a disabling course(47). However, biological markers were not analysed in that study. Since then, the role of biomarkers in predicting the disease course has been the focus of many studies, although their effectiveness in predicting outcomes vary(31,48–51). Most studies suggest that CRP predicts relapse in IBD(48–50), although one study found it had no predictive value (52). There are several reasons for this observed variation and includes differences in defining an aggressive disease course, disease heterogeneity and disease duration prior to analyses. It is also possible that variations in CRP genotype may explain variations in its performance in adult cohort studies. This has been described in the paediatric population(53), but yet to be explored in adults. The role of FC in predicting colectomy in acute severe colitis (ASUC) has been investigated previously (AUC 0.65, p=0.04) and more recently, SC has been shown to predict colectomy in ASUC with an AUC of 0.69 (95%CI 0.53-0.81) compared to FC (AUC 0.58; 95%CI 0.35-0.81) and CRP (AUC 0.71; 95%CI: 0.56-0.86) (16,38). SC has also been studied as a prognostic marker in predicting relapse after anti-TNF withdrawal and complements FC (>250µg/g) and hsCRP(>5mg/L) (p=0.0173, 0.0024 and 0.0002; HR: 3.191, 3.561 and 4.120 respectively) (17). Our study however is the first to explore the prognostic utility of SC at diagnosis. **Future studies incorporating periodic SC testing to predict disease course in IBD may be useful.**

Our study does have certain limitations. The results are from a single tertiary centre and based on a select cohort of newly diagnosed IBD patients. The relatively small numbers within the sub-type of IBD limits the power to dissect factors predicting phenotype and our diagnostic and prognostic models require further validation. **There were more females with functional bowel disease in the control cohort and this alone may underly the**

observation that male gender is a risk factor for IBD in our study. The major strengths of this study include a prospective design and a cohort of newly diagnosed IBD aiming for the first time to explore the correlation of SC with current biomarkers and build diagnostic and prognostic models for potential clinical use in IBD.

Conclusion

SC shows promise as a blood based biomarker in diagnosing and predicting disease course in IBD. A diagnostic and prognostic model that combines SC and other blood-based biomarkers accurately predicts the inflammatory burden in IBD and has the potential to predict disease and its outcomes. Our findings warrant further exploration and validation within large multicentre cohorts.

What is Current Knowledge

- Serum calprotectin (SC) has been studied as a prognostic marker in predicting relapse after anti-TNF withdrawal and complements FC and hsCRP for the prediction of relapse.
- SC can predict colectomy in acute severe colitis with an AUC of 0.69, comparable to CRP.
- SC correlates with other inflammatory blood markers such as CRP
- SC has been studied in diseases such as inflammatory arthropathies and cystic fibrosis and can predict disease progression.

What is New Here

- Serum Calprotectin (SC) is a strong individual predictor of a diagnosis of IBD
- **SC correlates with faecal calprotectin (FC) and is useful in diagnosis**
- SC can predict treatment escalation and/or surgery in IBD, in particular CD
- Blood based diagnostic and prognostic models can provide an accurate reflection of the inflammatory burden and have the potential to predict disease and its outcomes.

Competing interests

The study was supported by Calpro AS™, Norway who provided the ELISA kits for serum calprotectin testing.

Author contributions

Study design RK and JS. Patient recruitment and sample processing NTV, RK, NAK, RKB. Experimental work RK and MV. Data Analysis RK, NAK, ATA and NTV. RK wrote the manuscript. All authors were involved in critical review, editing, revision and approval of the final manuscript.

References

1. Kalla R, Ventham NT, Satsangi J, *et al.* Crohn's disease. BMJ 2014;349:g6670.
2. Ford AC, Moayyedi P, Hanauer SB. Ulcerative colitis. BMJ 2013;346:f432.
3. Molodecky NA, Soon IS, Rabi DM, *et al.* Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 2012;142:46–54.e42; quiz e30.
4. IBD standards group.Quality care: Service standards for the healthcare of people who have inflammatory bowel disease (IBD). 2009.
5. Loftus E V. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. Gastroenterology 2004;126:1504–1517.
6. Kappelman MD, Rifas-Shiman SL, Kleinman K, *et al.* The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. Clin. Gastroenterol. Hepatol. 2007;5:1424–9.
7. Kappelman MD, Rifas-Shiman SL, Porter CQ, *et al.* Direct Health Care Costs of Crohn's Disease and Ulcerative Colitis in US Children and Adults. Gastroenterology 2008;135:1907–1913.
8. Burisch J, Jess T, Martinato M, *et al.* The burden of inflammatory bowel disease in Europe. J. Crohns. Colitis 2013;7:322–37.
9. Sands BE. Biomarkers of Inflammation in Inflammatory Bowel Disease. Gastroenterology 2015;
10. Viennois E, Zhao Y, Merlin D. Biomarkers of Inflammatory Bowel Disease: From Classical Laboratory Tools to Personalized Medicine. Inflamm. Bowel Dis. 2015;21:2467–74.

11. P140. Proximity Extension Assay technology identifies novel serum biomarkers for predicting Inflammatory Bowel Disease: IBD Character Consortium. J. Crohns. Colitis 2015;9 Suppl 1:S146–7.

12. Kaiser T, Langhorst J, Wittkowski H, *et al.* Faecal S100A12 as a non-invasive marker distinguishing inflammatory bowel disease from irritable bowel syndrome. Gut 2007;56:1706–13.

13. Rheenen PF van, Vijver E Van de, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. BMJ 2010;341:c3369.

14. Lehmann FS, Burri E, Beglinger C. The role and utility of faecal markers in inflammatory bowel disease. Therap. Adv. Gastroenterol. 2015;8:23–36.

15. Wright EK, Kamm MA, Cruz P De, *et al.* Measurement of Fecal Calprotectin Improves Monitoring and Detection of Recurrence of Crohn's Disease Following Surgery. Gastroenterology 2015;

16. Ho GT, Lee HM, Brydon G, *et al.* Fecal calprotectin predicts the clinical course of acute severe ulcerative colitis. Am. J. Gastroenterol. 2009;104:673–678.

17. Meuwis M-A, Vernier-Massouille G, Grimaud JC, *et al.* Serum calprotectin as a biomarker for Crohn's disease. J. Crohns. Colitis 2013;7:e678–83.

18. Lasson A, Stotzer P-O, Ohman L, *et al.* The intra-individual variability of faecal calprotectin: A prospective study in patients with active ulcerative colitis. J. Crohns. Colitis 2014;

19. Calafat M, Cabré E, Mañosa M, *et al.* High within-day variability of fecal calprotectin levels in patients with active ulcerative colitis: what is the best timing for stool sampling? Inflamm. Bowel Dis. 2015;21:1072–6.

20. Reid PA, McAllister DA, Boyd AC, *et al.* Measurement of serum calprotectin in stable patients predicts exacerbation and lung function decline in cystic fibrosis. Am. J. Respir. Crit. Care Med. 2015;191:233–6.

21. Gray RD, Imrie M, Boyd AC, *et al.* Sputum and serum calprotectin are useful biomarkers during CF exacerbation. J. Cyst. Fibros. 2010;9:193–8.

22. Abildtrup M, Kingsley GH, Scott DL. Calprotectin as a Biomarker for Rheumatoid Arthritis: A Systematic Review. J. Rheumatol. 2015;

23. Obry A, Lequerré T, Hardouin J, *et al.* Identification of S100A9 as biomarker of responsiveness to the methotrexate/etanercept combination in rheumatoid arthritis using a proteomic approach. PLoS One 2014;9:e115800.

24. Mariani A, Marsili M, Nozzi M, *et al.* Serum calprotectin: review of its usefulness and validity in paediatric rheumatic diseases. Clin. Exp. Rheumatol. 2014;33:109–114.

25. Hammer HB, Ødegård S, Syversen SW, *et al.* Calprotectin (a major S100 leucocyte protein) predicts 10-year radiographic progression in patients with rheumatoid arthritis. Ann. Rheum. Dis. 2010;69:150–4.

26. Boschetti G, Garnero P, Moussata D, *et al.* Accuracies of Serum and Fecal S100 Proteins (Calprotectin and Calgranulin C) to Predict the Response to TNF Antagonists in Patients with Crohn's Disease. Inflamm. Bowel Dis. 2015;21:331–6.

27. Cury DB, Mizsputen SJ, Versolato C, *et al.* Serum calprotectin levels correlate with biochemical and histological markers of disease activity in TNBS colitis. Cell. Immunol. 2013;282:66–70.

28. Silverberg MS, Satsangi J, Ahmad T, *et al.* Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can. J.

458 Gastroenterol. 2005;19 Suppl A:5A–36A.

459 29. Lennard-Jones JE. Classification of inflammatory bowel disease. Scand. J.
460 Gastroenterol. Suppl. 1989;170:2–6; discussion 16–9.

461 30. Levine A, Griffiths A, Markowitz J, *et al.* Pediatric modification of the Montreal
462 classification for inflammatory bowel disease: the Paris classification. Inflamm. Bowel
463 Dis. 2011;17:1314–21.

464 31. Lee JC, Lyons P a, McKinney EF, *et al.* Gene expression profiling of CD8+ T cells
465 predicts prognosis in patients with Crohn disease and ulcerative colitis. J. Clin. Invest.
466 2011;121:4170–9.

467 32. Robin X, Turck N, Hainard A, *et al.* pROC: an open-source package for R and S+ to
468 analyze and compare ROC curves. BMC Bioinformatics 2011;12:77.

469 33. Satsangi J, Silverberg MS, Vermeire S, *et al.* The Montreal classification of
470 inflammatory bowel disease: controversies, consensus, and implications. Gut
471 2006;55:749–53.

472 34. Averill MM, Kerkhoff C, Bornfeldt KE. S100A8 and S100A9 in cardiovascular
473 biology and disease. Arterioscler. Thromb. Vasc. Biol. 2012;32:223–9.

474 35. Ingersoll MA, Spanbroek R, Lottaz C, *et al.* Comparison of gene expression profiles
475 between human and mouse monocyte subsets. Blood 2010;115:e10–9.

476 36. Fagerhol MK, Nielsen HG, Vetlesen A, *et al.* Increase in plasma calprotectin during
477 long-distance running. 2005.

478 37. Leach ST, Yang Z, Messina I, *et al.* Serum and mucosal S100 proteins, calprotectin
479 (S100A8/S100A9) and S100A12, are elevated at diagnosis in children with
480 inflammatory bowel disease. Scand. J. Gastroenterol. 2007;42:1321–31.

38. Hare NC, Kennedy NA, Kalla R, *et al.* P349 Serum calprotectin: a novel biomarker to predict outcome in acute severe ulcerative colitis? J. Crohn's Colitis 2014;8:S210.

39. Mor G, Visintin I, Lai Y, *et al.* Serum protein markers for early detection of ovarian cancer. Proc. Natl. Acad. Sci. U. S. A. 2005;102:7677–82.

40. Xiao T, Ying W, Li L, *et al.* An approach to studying lung cancer-related proteins in human blood. Mol. Cell. Proteomics 2005;4:1480–6.

41. Kennedy NA, Clark A, Walkden A, *et al.* Clinical utility and diagnostic accuracy of faecal calprotectin for IBD at first presentation to gastroenterology services in adults aged 16-50 years. J. Crohns. Colitis 2015;9:41–9.

42. Mosli MH, Zou G, Garg SK, *et al.* C-Reactive Protein, Fecal Calprotectin, and Stool Lactoferrin for Detection of Endoscopic Activity in Symptomatic Inflammatory Bowel Disease Patients: A Systematic Review and Meta-Analysis. Am. J. Gastroenterol. 2015;110:802–819.

43. af Björkesten C-G, Nieminen U, Turunen U, *et al.* Surrogate markers and clinical indices, alone or combined, as indicators for endoscopic remission in anti-TNF-treated luminal Crohn's disease. Scand. J. Gastroenterol. 2012;47:528–37.

44. D'Haens G, Ferrante M, Vermeire S, *et al.* Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm. Bowel Dis. 2012;18:2218–24.

45. Schoepfer AM, Beglinger C, Straumann A, *et al.* Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. Am. J. Gastroenterol. 2010;105:162–9.

46. Langhorst J, Elsenbruch S, Koelzer J, *et al.* Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal

lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. Am. J. Gastroenterol. 2008;103:162–9.

47. Beaugerie L, Seksik P, Nion-Larmurier I, *et al.* Predictors of crohn's disease. Gastroenterology 2006;130:650–656.

48. Boirivant M, Leoni M, Tariciotti D, *et al.* The clinical significance of serum C reactive protein levels in Crohn's disease. Results of a prospective longitudinal study. J. Clin. Gastroenterol. 1988;10:401–405.

49. Niewiadomski O, Studd C, Hair C, *et al.* Prospective population-based cohort of inflammatory bowel disease in the biologics era: Disease course and predictors of severity. J. Gastroenterol. Hepatol. 2015;30:1346–53.

50. Henriksen M, Jahnsen J, Lygren I, *et al.* C-reactive protein: a predictive factor and marker of inflammation in inflammatory bowel disease. Results from a prospective population-based study. Gut 2008;57:1518–1523.

51. Sandor Kiss L, Papp M, Dorottya Lovasz B, *et al.* High-sensitivity C-reactive protein for identification of disease phenotype, active disease, and clinical relapses in Crohn's disease: A marker for patient classification? Inflamm. Bowel Dis. 2012;18:1647–1654.

52. Costa F, Mumolo MG, Ceccarelli L, *et al.* Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. Gut 2005;54:364–368.

53. Henderson P, Kennedy NA, Limbergen JE Van, *et al.* Serum C-reactive protein and CRP genotype in pediatric inflammatory bowel disease: influence on phenotype, natural history, and response to therapy. Inflamm. Bowel Dis. 2015;21:596–605.

Figure Legends

Figure 1: **Serum Calprotectin levels in patients with Crohn's disease (CD), Ulcerative colitis (UC), Inflammatory bowel disease unclassified (IBDU), symptomatic controls (non-IBD) and healthy controls (HC)**

Footnote: Boxplots represent median and inter-quartile ranges for serum calprotectin within each subcohort

Figure 2: Receiver operating curve analysis (ROC) of serum calprotectin (SC) and other blood based markers in differentiating Inflammatory bowel diseases (IBD) from non-IBD and ROC analysis of SC and faecal calprotectin (FC) (within 30 days) in discriminating Inflammatory bowel diseases (IBD) from non-IBD.

Footnote: WCC:white cell count; CRP:C-reactive protein; Hb:Haemoglobin

Figure 3a: Kaplan Meier survival curves of disease course using blood biomarkers to predict outcomes in newly diagnosed Inflammatory Bowel Diseases (IBD). Single marker represents either or albumin<37 g/L or serum calprotectin ≥ 1046ng/ml. Dual markers represents a combination of both variables.

Figure 3b: Kaplan Meier survival curves of disease course using blood biomarkers to predict outcomes in newly diagnosed Crohn's disease (CD)

Footnote: '1 marker' represents either CRP>24mg/L or albumin<26 g/L or serum calprotectin >991 ng/ml. '2 or 3 marker' represents a combination of any 2 or 3 of the above mentioned variables.

549 **Figure 3c**: Kaplan Meier survival curves of disease course using blood biomarkers to predict
550 outcomes in newly diagnosed Ulcerative Colitis (UC)

551 **Footnote**: Single marker represents either albumin<37 g/L or CRP >2.5mg/L. Dual markers
552 represents all the categorical variables as a combined biomarker.

553 **Figure 4**: Correlation between log transformed serum calprotectin and neutrophil count in
554 Inflammatory Bowel Diseases (IBD) versus controls (Non-IBD).

Table 1: Correlation coefficient (spearman rho) between Serum Calprotectin (SC) and blood and faecal parameters

Parameters	Number of patients	rho(spearman rho)	P value
SC – CRP	171	0.61	$6.9x10^{-19}$
SC – WCC	147	0.61	3.8×10^{-17}
SC – Neut	147	0.65	$1.9x10^{-20}$
SC – Lymp	147	-0.03	0.68
SC – Alb	171	-0.54	$3.3x10^{-14}$
SC-Hb	147	-0.42	6.1×10^{-8}
SC-Plts	147	0.49	1.3×10^{-10}
SC-FC	50	0.50	$1.6x10^{-4}$

Parameters	IBD(n)	rho(p-value)	Non-IBD(n)	rho(p-value)
SC – CRP	96	$0.41(3.9x10^{-5})$	75	0.30(0.01)
SC – WCC	92	$0.37(2.3x10^{-4})$	55	$0.66(4.3x10^{-8})$
SC - Neut	92	$0.43(2.2x10^{-5})$	55	$0.68(1.0x10^{-8})$
SC – Lymp	92	-0.18(0.08)	55	$0.48(2.0x10^{-4})$
SC – Alb	95	$-0.39(6.9x10^{-5})$	75	-0.09(0.46)
SC-Hb	92	$-0.41(4.7x10^{-5})$	55	-0.05(0.75)
SC-Plts	92	0.31 (0.002)	55	0.27(0.04)
SC-FC	31	-0.07(0.72)	19	0.13(0.59)

Abbreviations: SC: serum calprotectin, WCC: white cell count; Neut: neutrophil count; Lymp: lymphocyte count; Alb: albumin; Hb: haemoglobin; FC: faecal calprotectin; Plts: Platelet count

Table 2: Study demographics, Montreal classification, disease behaviour for newly diagnosed Inflammatory Bowel Diseases (IBD) and control cohorts

Variables	Inflammatory Bowel Diseases (n=83)	Controls (n=73)
Subtype IBD(CD:UC:IBDU)	35:45:3	
Subtype control group(HC:IBS)		27:46
Males (%)	58(69)	33(45)
Smoking status (current: never: ex)	14:41:26	12:36:15
Median age (range)	31(18-73)	31(19-64)
Montreal classification for CD		
L1 +/-L4	10	
L2 +/-L4	10	
L3 +/-L4	15	
Montreal behaviour for CD		
B1	21	
B2	2	
B3	6	
Not available	6	
Paris Extent for UC		
E1	5	
E2	14	
E3	10	
E4	15	
Not available	1	

Footnote: CD: Crohn's disease; UC: ulcerative colitis; IBD-U: Inflammatory bowel disease unclassified; HC: healthy controls; SC: symptomatic controls.

Smoking status was available in 81 patients with IBD and 63 patients with non-IBD.

Table 3: Blood and faecal parameters for Inflammatory Bowel Disease (IBD) and control cohorts

	IBD		Controls	
Test	Number of patients	Median (IQR)	Number of patients	Median (IQR)
Haemoglobin (g/L)	79	128(119-139)	53	140(135-150)
Neutrophil (10^9/L)	79	5.8(3.9-7.5)	53	3.2(2.5-4.1)
Lymphocyte (mg/L)	79	1.7(1.2-2.1)	53	1.8(1.5-2.2)
White cell count(10^9/L)	79	8.3(6.5-10.7)	53	5.8(4.7-6.6)
Platelet count (10^9/L)	79	335(269-432)	53	244(209-277)
C-reactive protein(mg/L)	83	11(3.0-35.5)	73	1.0(0.5-2.0)
Faecal calprotectin within 30 days (µg/g)	31	770(660-880)	19	19(19-25)
Albumin (g/L)	83	35(29.0-39.0)	73	42(39-46)
Serum Calprotectin (ng/ml)	83	1015(811-1442)	73	506(362-725)

Table 4: Multiple logistic regression of predictors of Inflammatory Bowel Diseases versus controls (n=155)

Continuous variable analysis		
Variable	**Odds ratio (95%CI)**	**P value**
Log(C-reactive protein)	6.60 (2.18-23.67)	0.002
Log(Serum Calprotectin)	296.85 (9.55-18512.49)	0.003
Albumin	0.85 (0.75-0.94)	0.003
Gender	4.00(1.22-14.68)	0.03

Categorical variable analysis		
Categorical threshold	**Odds ratio (95%CI)**	**P-value for thresholds**
C-reactive protein>3.5mg/L	8.52(2.75-28.63)	2.80×10^{-4}
Serum Calprotectin >852ng/ml	9.37(2.82-34.68)	4.00×10^{-4}
Albumin<38g/L	6.12 (1.82-22.16)	0.004
Male gender	2.87(0.97-9.24)	0.06

Table 5a: Sensitivity, specificity, positive and negative likelihood ratios (LR) of the Inflammatory Bowel Diseases (IBD) scoring parameters. Each variable score is based on the odds ratio generated from the linear model

Variable	Score
Serum Calprotectin>852ng/ml	5
Albumin <38g/L	3
CRP≥3.5mg/L	4
Male gender	1

IBD Score	Sensitivity	Specificity	Positive LR	Negative LR
1 or above	0.96	0.31	1.39	0.12
3 or above	0.89	0.68	2.79	0.16
4 or above	0.85	0.75	3.41	0.20
5 or above	0.84	0.89	7.57	0.18
6 or above	0.74	0.93	10.71	0.28
7 or above	0.68	0.96	16.39	0.33
8 or above	0.67	0.97	24.14	0.34

Table 5b: Sensitivity, specificity, positive and negative likelihood ratios (LR) of the Inflammatory Bowel Diseases (IBD) of the individual markers.

Test	Sensitivity	Specificity	Positive LR	Negative LR
C-reactive protein>3.5mg/L	0.70	0.86	5.03	0.35
Albumin<38 g/L	0.66	0.88	5.30	0.39
Serum Calprotectin>852ng/ml	0.69	0.90	7.06	0.35

Table 6a: Multivariable analysis for predictive factors for an aggressive disease course in patients with Inflammatory Bowel Diseases (n=83): final Cox proportional hazards model

Categorical variable analysis				
Categorical variable	**Categorical threshold**	**Hazards ratio (95%CI)**	**AIC**	**P-value for thresholds**
Serum Calprotectin(SC)	≥1046 ng/ml	2.7(1.3-5.6)	309.2	0.007
Albumin	<37 g/L	2.5(1.1-5.6)	306.5	0.03

Table 6b: Multivariable analysis for predictive factors for an aggressive disease course in patients with Ulcerative Colitis (n=45): final Cox proportional hazards model

	Categorical variable analysis			
Categorical variable	**Categorical threshold**	**Hazards ratio (95%CI)**	**AIC**	**P-value for thresholds**
Albumin	<37 g/L	3.8(1.2-11.9)	148.0	0.02
C-reactive protein(CRP)	>2.5mg/L	2.6(0.7-9.6)	147.0	0.15

Table 6c: Multivariable analysis for predictive factors for an aggressive disease course in patients with Crohn's disease (n=35): final Cox proportional hazards model

Categorical variable analysis				
Categorical variable	**Categorical threshold**	**Hazards ratio (95%CI)**	**AIC**	**P-value for thresholds**
C-reactive protein(CRP)	>24mg/L	2.7(1.0-7.4)	93.3	0.06
Albumin	<26 g/L	2.6(0.8-9.1)	91.9	0.13
Serum Calprotectin	>991ng/ml	4.2(1.2-15.3)	95.1	0.03

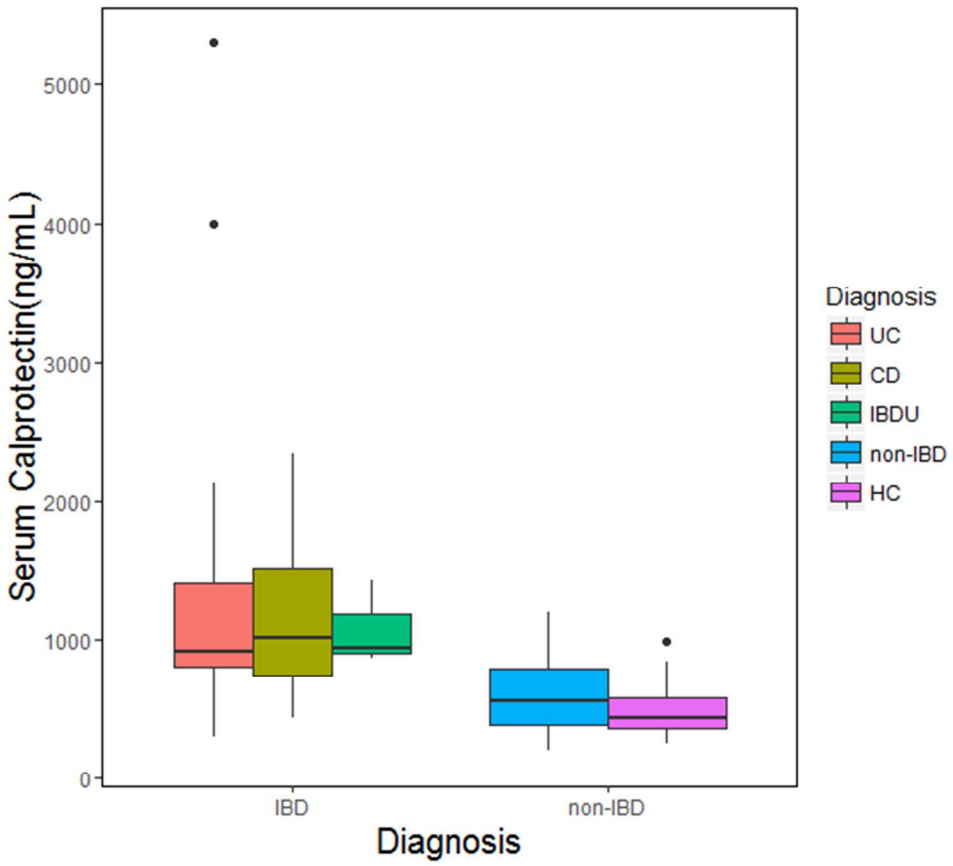

Figure 1: Serum Calprotectin levels in patients with Crohn's disease (CD), Ulcerative colitis (UC), Inflammatory bowel disease unclassified (IBDU), symptomatic controls (non-IBD) and healthy controls (HC)

Footnote: Boxplots represent median and inter-quartile ranges for serum calprotectin within each subcohort

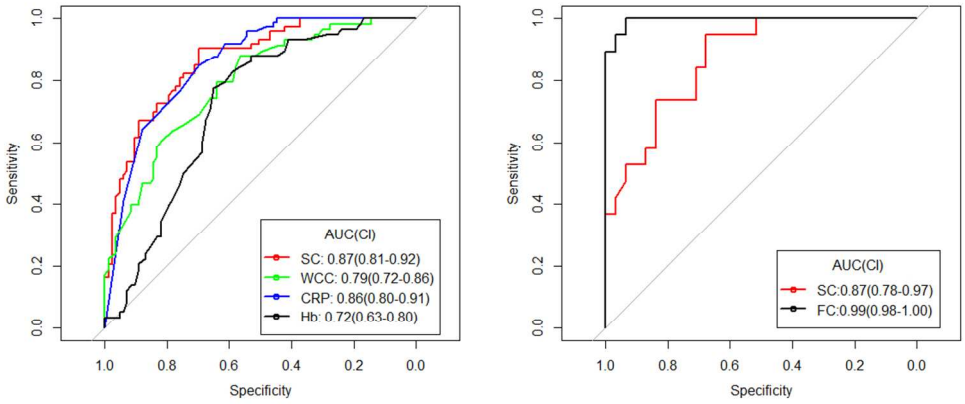

Figure 2: Receiver operating curve analysis (ROC) of serum calprotectin (SC) and other blood based markers in differentiating Inflammatory bowel diseases (IBD) from non-IBD and ROC analysis of SC and faecal calprotectin (FC) (within 30 days) in discriminating Inflammatory bowel diseases (IBD) from non-IBD.
Footnote: WCC:white cell count; CRP:C-reactive protein; Hb:Haemoglobin

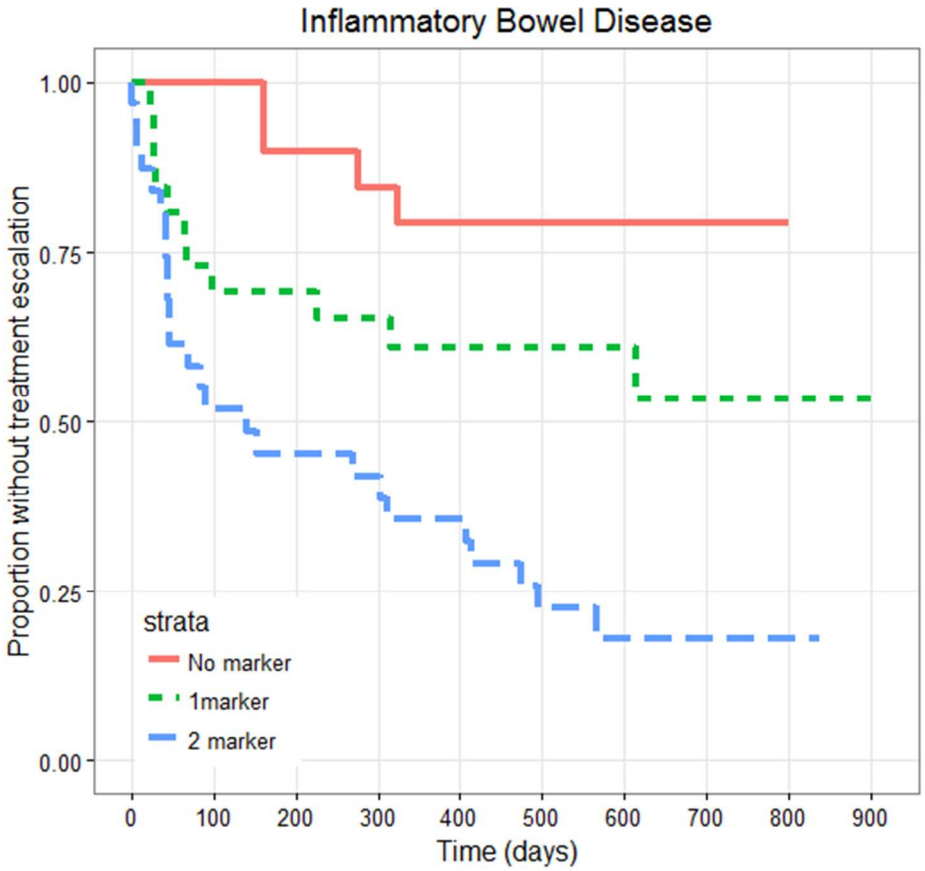

Figure 3a: Kaplan Meier survival curves of disease course using blood biomarkers to predict outcomes in newly diagnosed Inflammatory Bowel Diseases (IBD). Single marker represents either or albumin<37 g/L or serum calprotectin ≥ 1046ng/ml. Dual markers represents a combination of both variables.

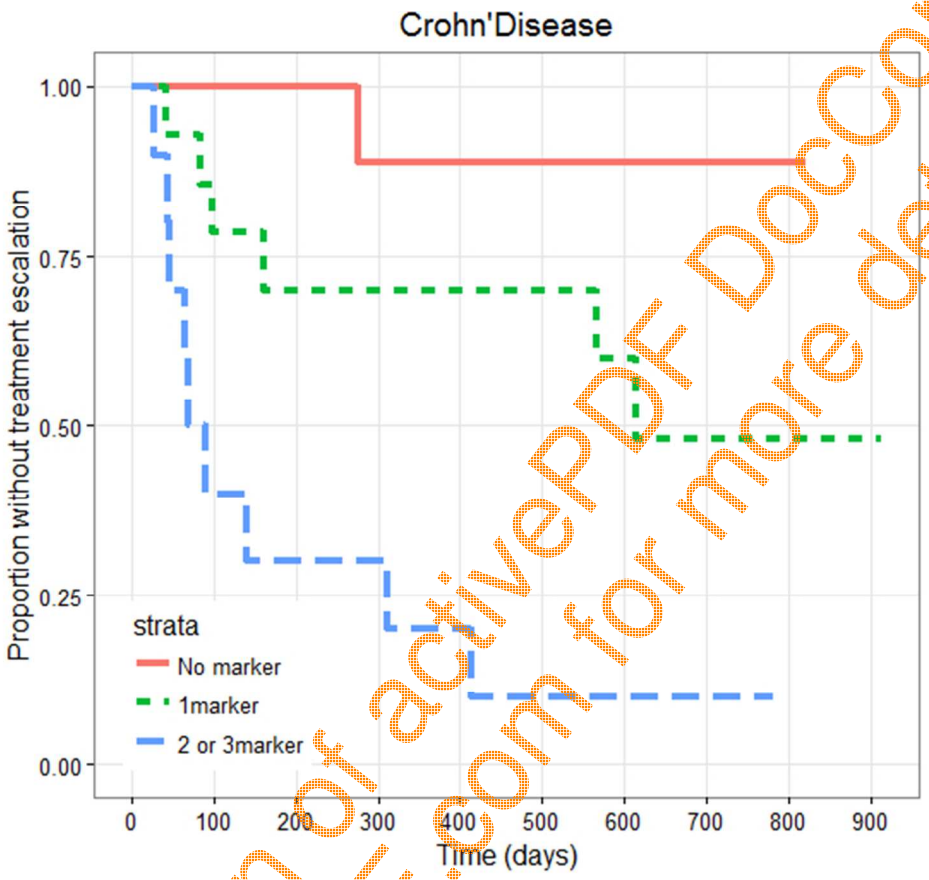

Figure 3b: Kaplan Meier survival curves of disease course using blood biomarkers to predict outcomes in newly diagnosed Crohn's disease (CD)

Footnote: '1 marker' represents either CRP>24mg/L or albumin<26 g/L or serum calprotectin >991 ng/ml. '2 or 3 marker' represents a combination of any 2 or 3 of the above mentioned variables.

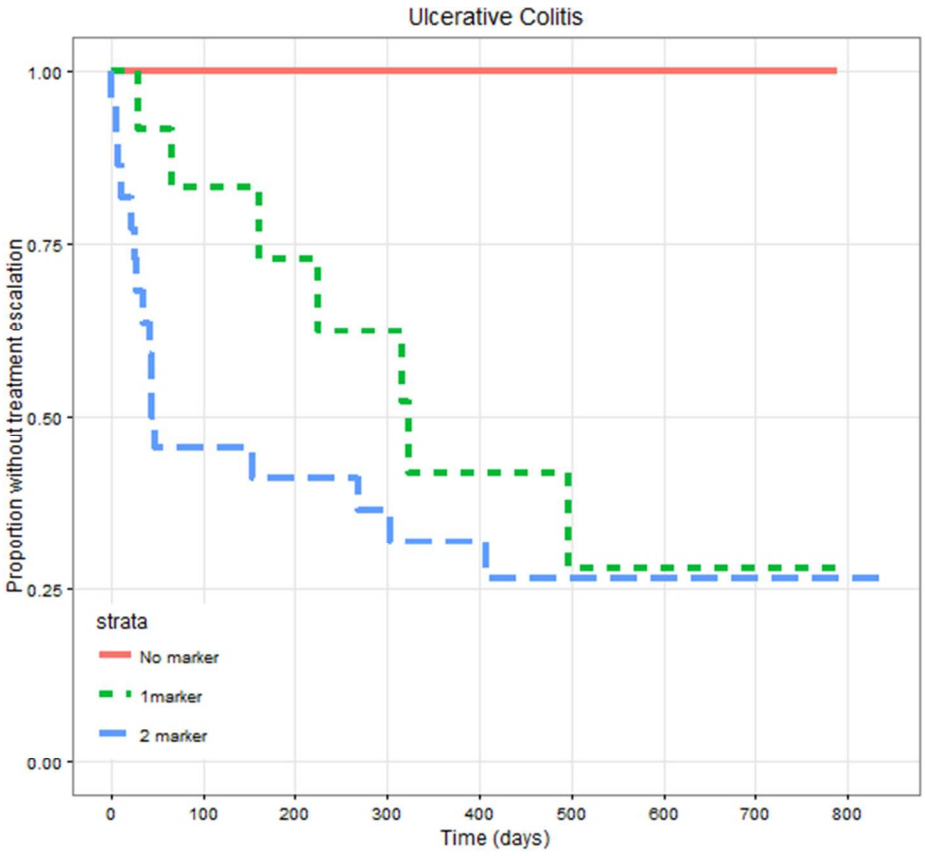

Figure 3c: Kaplan Meier survival curves of disease course using blood biomarkers to predict outcomes in newly diagnosed Ulcerative Colitis (UC)
Footnote: Single marker represents either albumin<37 g/L or CRP >2.5mg/L. Dual markers represents all the categorical variables as a combined biomarker.

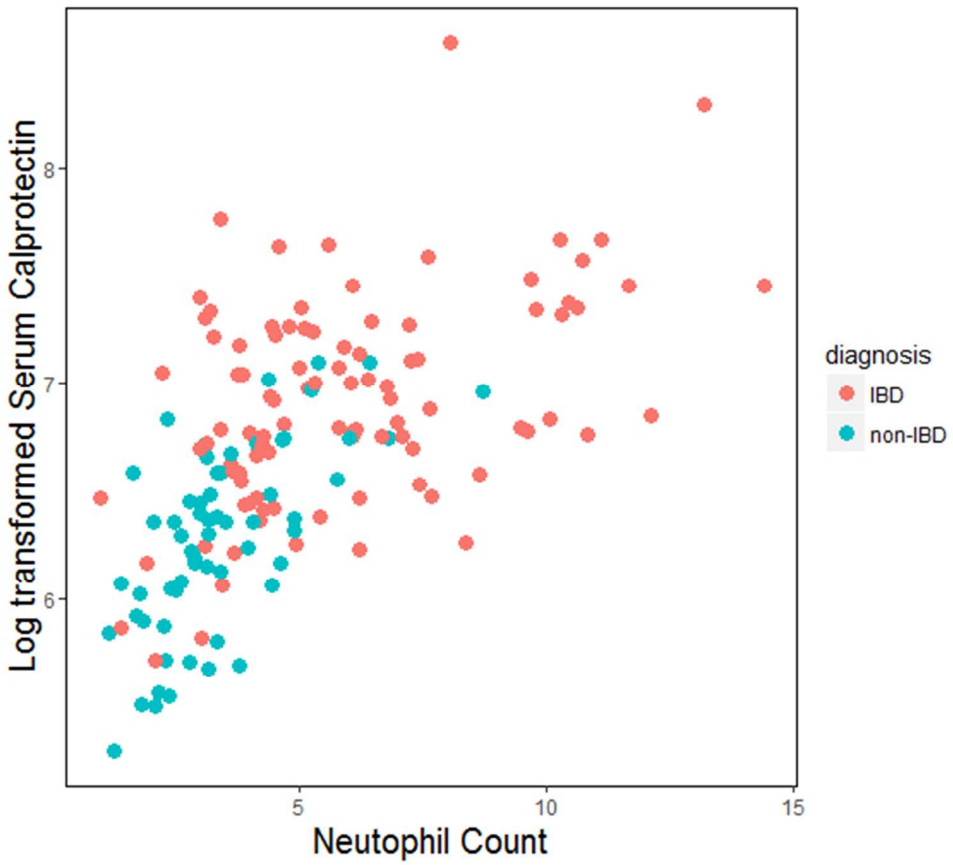

Figure 4: Correlation between log transformed serum calprotectin and neutrophil count in Inflammatory Bowel Diseases (IBD) versus controls (Non-IBD).

Supplementary Table 1: Disease outcomes in patients with Ulcerative colitis (UC) and Crohn's disease (CD).

Diagnosis	Treatment Escalation?	Age	Sex	Total follow up time (days)	Diagnosis to SC sampling (days)	SC sampling to Rx escalation(days)	Rx immediately prior escalation	Treatment escalation
UC	Yes	46	M	126	1	30	Prednisolone	Azathioprine and 2nd course of Prednisolone
UC	Yes	54	M	374	73	27	5ASA	Azathioprine and Prednisolone
UC	Yes	35	F	547	-14	316	5ASA	Azathioprine and Prednisolone
UC	Yes	33	F	609	18	67	5ASA	Azathioprine and Prednisolone
UC	Yes	53	M	871	2	47	5ASA	Azathioprine and Prednisolone
UC	Yes	20	M	893	-14	269	5ASA	Azathioprine and Prednisolone
UC	Yes	29	M	201	0	44	Prednisolone	Ciclosporin and Azathioprine during 2nd admission
UC	Yes	19	M	470	2	43	5ASA	Ciclosporin, Prednisolone and Azathioprine
UC	Yes	31	M	781	-1	408	5ASA	Ciclosporin, Prednisolone and Azathioprine
UC	Yes	34	M	498	37	225	Azathioprine	Golilumab
UC	Yes	33	F	69	41	26	Azathioprine	Infliximab
UC	Yes	26	F	549	8	44	Azathioprine	Infliximab
UC	No	35	M	118	0	NA	5ASA	No change in therapy
UC	No	38	F	132	43	NA	5ASA	No change in therapy
UC	No	32	M	145	0	NA	5ASA	No change in therapy
UC	No	32	M	145	0	NA	5ASA	No change in therapy
UC	No	53	M	345	12	NA	5ASA	No change in therapy
UC	No	40	M	367	24	NA	5ASA	No change in therapy
UC	No	24	F	410	0	NA	5ASA	No change in therapy
UC	No	27	F	456	57	NA	5ASA	No change in therapy
UC	No	37	F	472	78	NA	5ASA	No change in therapy
UC	No	48	M	484	68	NA	5ASA	No change in therapy
UC	No	41	M	499	0	NA	Azathioprine	No change in therapy
UC	No	57	M	518	23	NA	5ASA	No change in therapy
UC	No	27	M	575	-39	NA	5ASA	No change in therapy
UC	No	24	F	578	0	NA	5ASA	No change in therapy

UC	No	58	M	604	75	NA	5ASA	No change in therapy
UC	No	50	M	637	61	NA	5ASA	No change in therapy
UC	No	62	M	657	32	NA	5ASA	No change in therapy
UC	No	26	M	670	30	NA	No medications	No change in therapy
UC	No	57	F	788	45	NA	5ASA	No change in therapy
UC	No	38	M	795	27	NA	5ASA	No change in therapy
UC	No	52	M	801	78	NA	5ASA	No change in therapy
UC	No	34	M	837	-1	NA	Azathioprine	No change in therapy
UC	Yes	29	M	366	0	7	IVMP and Ciclosporin	Panproctocolectomy
UC	Yes	44	M	451	2	6	MP and Prednisolone	Panproctocolectomy
UC	Yes	21	M	126	13	35	Prednisolone and Ciclosporin	Subtotal colectomy
UC	Yes	59	M	433	5	1	5ASA	Subtotal colectomy
UC	Yes	43	F	602	25	323	MP	Subtotal colectomy
UC	Yes	22	M	643	22	303	5ASA	Subtotal colectomy
UC	Yes	27	F	672	4	153	5ASA	Subtotal colectomy
UC	Yes	22	M	712	0	23	5ASA	Subtotal colectomy
UC	Yes	23	F	871	1	496	5ASA and Azathioprine	Subtotal colectomy
UC	Yes	29	M	385	-12	12	Prednisolone and Ciclosporin	Subtotal colectomy
UC	Yes	39	M	342	0	161	MP	Vedolizumab
CD	Yes	42	M	602	1	413	Azathioprine	Adalimumab
CD	Yes	27	M	852	0	311	Azathioprine	Adalimumab
CD	Yes	27	M	870	0	614	MP	Adalimumab
CD	Yes	18	M	358	44	275	Infliximab	Azathioprine
CD	Yes	24	F	510	0	47	Infliximab	Azathioprine
CD	Yes	25	M	454	0	91	Prednisolone	Azathioprine and 2nd course Prednisolone
CD	Yes	19	M	525	35	27	Prednisolone	Azathioprine and Infliximab
CD	Yes	23	M	504	34	69	5ASA	Azathioprine and Prednisolone
CD	Yes	27	F	644	11	566	5ASA	Azathioprine and Prednisolone
CD	Yes	27	F	762	50	84	MP and Infliximab	Ileocaecal resection
CD	No	29	M	768	-2	6	IVMP	Ileocaecal resection at index admission

CD	Yes	32	M	618	30	161	MP	Infliximab
CD	Yes	19	F	664	20	45	MP	Infliximab
CD	Yes	33	F	718	1	42	Azathioprine	Infliximab
CD	Yes	30	M	803	-27	64	Azathioprine	Infliximab
CD	Yes	24	M	837	0	140	Azathioprine	Infliximab
CD	No	41	M	150	-4	NA	Azathioprine	No change in therapy
CD	No	59	F	160	-2	NA	Polymeric diet	No change in therapy
CD	No	31	M	177	-113	NA	No medications	No change in therapy
CD	No	72	M	347	46	NA	No medications	No change in therapy
CD	No	29	M	408	52	NA	No medications	No change in therapy
CD	No	38	M	417	87	NA	No medications	No change in therapy
CD	No	29	M	506	-18	NA	Azathioprine	No change in therapy
CD	No	52	M	513	57	NA	5ASA	No change in therapy
CD	No	31	M	555	0	NA	Azathioprine	No change in therapy
CD	No	29	F	582	-36	NA	Azathioprine	No change in therapy
CD	No	18	M	637	28	NA	No medications	No change in therapy
CD	No	57	F	719	33	NA	5ASA	No change in therapy
CD	No	40	F	721	18	NA	Azathioprine	No change in therapy
CD	No	23	M	726	22	NA	No medications	No change in therapy
CD	No	19	M	781	-6	NA	Azathioprine	No change in therapy
CD	No	38	F	821	64	NA	No medications	No change in therapy
CD	No	36	F	914	76	NA	5ASA	No change in therapy
CD	Yes	21	M	504	4	99	MP	Subtotal colectomy
CD	Yes	22	F	319	-206	NA	Azathioprine	No change in therapy

Footnote: F: Female; M: Male; NA: Not applicable; 5ASA: 5-aminosalicylates; MP: mercaptopurine; IVMP: Intravenous methylprednisolone

Supplementary Table 2a: Clinical symptoms (Simple Clinical Colitis Activity Index) at recruitment in patients with Ulcerative Colitis (UC) and Inflammatory Bowel Disease-Unclassified (IBDU)

Diagnosis	Rx naive	General Well Being	Complications	SCCAI Day BO	SCCAI Night BO	SCCAI Urgency	SCCAI Blood PR	SCCAI Score
UC	Y	2	0	0	0	1	3	6
UC	N	1	1	0	0	1	2	5
UC	N	1	0	1	1	NA	3	NA
UC	Y	1	0	1	0	1	0	3
UC	Y	1	0	0	0	1	2	4
UC	N	0	1	0	0	0	0	1
UC	Y	1	0	0	0	0	2	3
UC	Y	1	0	2	1	1	3	8
UC	Y	1	0	2	1	0	2	6
UC	Y	2	0	3	1	1	3	10
UC	Y	3	1	1	1	1	2	9
UC	N	2	0	2	1	1	3	9
UC	N	0	0	0	0	1	3	4
UC	N	0	0	0	0	0	0	0
UC	N	0	0	1	0	1	2	4
UC	N	1	0	2	1	1	2	7
UC	N	1	0	0	0	0	2	3
UC	N	0	0	0	1	2	0	3
UC	Y	2	0	3	2	1	3	11
UC	Y	1	0	3	0	3	3	10
UC	N	1	0	1	0	1	3	6
UC	N	1	0	1	2	1	3	8
UC	Y	2	0	0	2	1	2	7
UC	Y	1	0	2	0	1	3	7
UC	N	NA	NA	NA	NA	NA	NA	NA
UC	N	1	0	3	1	1	3	9
UC	Y	1	0	0	0	1	0	2
UC	N	1	0	3	2	1	3	10
UC	Y	1	0	0	0	1	2	4

UC	N	2	0	0	1	1	2	6
UC	N	4	0	3	2	3	3	15
UC	Y	2	0	3	2	3	3	13
UC	Y	1	0	2	1	1	2	7
UC	Y	1	1	0	0	0	3	5
UC	N	0	0	0	0	1	3	4
UC	Y	2	0	NA	NA	1	3	NA
UC	N	1	0	0	0	0	1	2
UC	Y	1	1	0	0	1	2	5
UC	Y	1	0	2	0	1	3	7
UC	Y	1	1	1	0	1	2	6
UC	N	1	1	0	0	1	2	5
UC	N	1	1	0	1	1	2	6
UC	N	1	0	0	0	2	3	6
UC	Y	3	0	1	1	1	2	8
UC	N	2	0	1	2	2	3	10
IBDU	Y	0	0	0	0	0	2	2
IBDU	N	1	0	1	0	1	0	3
IBDU	Y	2	0	1	1	2	3	9

Supplementary Table 2b: Clinical symptoms (Harvey Bradshaw Index) at recruitment in patients with Crohn's Disease (CD)

Diagnosis	Rx naive	Weight loss	General Well Being	Abdominal Pain	BO per day	Abdominal mass	Complications	HBI Score
CD	Y	N	0	0	2	NA	0	NA
CD	N	N	NA	1	11	0	1	NA
CD	Y	Y	1	0	1	0	1	3
CD	Y	Y	1	0	1	0	0	2
CD	N	Y	2	1	10	0	1	14
CD	Y	N	1	3	9	0	0	13
CD	Y	Y	2	2	5	1	0	10
CD	Y	N	1	1	1	2	0	5
CD	Y	Y	2	2	0	1	0	5
CD	Y	Y	0	0	1	0	0	1
CD	Y	Y	2	2	1	0	1	6
CD	Y	Y	0	0	1	0	0	1
CD	N	N	1	0	11	0	0	12
CD	Y	Y	NA	3	10	0	1	NA
CD	Y	Y	1	1	2	0	0	4
CD	Y	Y	1	0	1	0	1	3
CD	Y	Y	NA	NA	NA	NA	NA	NA
CD	Y	N	0	2	1	3	0	6
CD	N	Y	1	1	2	0	1	5
CD	Y	N	1	1	2	0	2	6
CD	Y	Y	1	2	4	0	0	7
CD	N	Y	1	3	2	0	2	8
CD	N	Y	1	0	5	0	1	7
CD	Y	Y	2	2	10	0	2	16
CD	Y	Y	1	2	7	0	1	11
CD	Y	Y	1	1	10	0	0	12
CD	Y	Y	2	2	8	0	0	12
CD	Y	N	2	0	5	0	1	8
CD	Y	N	2	2	7	NA	2	NA

CD	Y	N	0	0	0	0	2	2
CD	Y	NA	NA	0	1	0	0	NA
CD	Y	NA	1	3	3	0	2	9
CD	Y	N	1	1	2	0	0	4
CD	Y	N	0	0	4	0	0	4
CD	Y	Y	1	3	2	0	0	6

Footnote: Rx naïve: Treatment naïve status at sampling; SC: serum calprotectin; BO: Bowel habits; SCCAI: Simple clinical colitis activity index; HBI: Harvey Bradshaw Index; NA: Not available; Y: yes; N: No

Supplementary Table 3: Summary of investigations undertaken in the control cohort

Diagnosis	Age	Sex	Faecal Calprotectin (µg/g)	Colonoscopy	Radiology
HC	47	M	NA	no	no
HC	42	M	NA	no	no
HC	32	M	NA	no	no
HC	30	M	NA	no	no
HC	31	F	NA	no	no
HC	24	F	NA	no	no
HC	33	M	NA	no	no
HC	34	F	NA	no	no
HC	32	F	NA	no	no
HC	30	M	NA	no	no
HC	32	F	NA	no	no
HC	54	F	NA	no	no
HC	39	M	NA	no	no
HC	43	M	NA	no	no
HC	59	F	NA	no	no
HC	35	F	NA	no	no
HC	25	F	NA	no	no
HC	20	F	NA	no	no
HC	31	M	NA	no	no
HC	19	F	NA	no	no

HC	64	F	NA	no	no
HC	49	F	NA	no	no
HC	24	M	NA	no	no
HC	30	M	NA	no	no
HC	30	M	NA	no	no
HC	33	M	NA	no	no
HC	34	M	NA	no	no
Non-IBD	31	F	19	no	no
Non-IBD	22	M	19	yes	no
Non-IBD	33	F	19	no	yes
Non-IBD	21	M	19	yes	yes
Non-IBD	31	M	19	yes	no
Non-IBD	21	M	19	no	no
Non-IBD	29	F	19	yes	no
Non-IBD	37	F	19	yes	no
Non-IBD	28	M	NA	yes	no
Non-IBD	28	F	NA	yes	no
Non-IBD	33	F	NA	yes	no
Non-IBD	27	F	19	no	no
Non-IBD	23	F	30	yes	yes
Non-IBD	33	F	19	no	no
Non-IBD	29	F	NA	yes	no
Non-IBD	23	F	19	yes	no
Non-IBD	44	F	NA	yes	no
Non-IBD	41	M	NA	no	yes
Non-IBD	28	F	120	yes	yes
Non-IBD	26	F	19	yes	yes
Non-IBD	33	M	19	yes	no
Non-IBD	41	F	19	no	no
Non-IBD	19	F	19	yes	yes

Non-IBD	22	F	19	yes	yes
Non-IBD	29	F	30	yes	no
Non-IBD	39	F	19	yes	yes
Non-IBD	34	F	210	yes	yes
Non-IBD	28	F	80	yes	no
Non-IBD	23	M	19	yes	yes
Non-IBD	29	F	NA	yes	no
Non-IBD	21	M	19	yes	no
Non-IBD	22	M	19	no	no
Non-IBD	44	M	470	yes	yes
Non-IBD	39	M	19	yes	no
Non-IBD	32	M	NA	yes	no
Non-IBD	30	F	160	yes	no
Non-IBD	37	F	190	yes	no
Non-IBD	36	M	NA	yes	no
Non-IBD	34	F	110	yes	yes
Non-IBD	43	M	NA	yes	no
Non-IBD	45	M	NA	yes	no
Non-IBD	23	M	19	no	no
Non-IBD	41	M	NA	yes	yes
Non-IBD	21	M	19	no	no
Non-IBD	20	F	19	yes	no
Non-IBD	20	F	19	yes	no

Footnote: HC: Healthy lab volunteers; Non-IBD: Symptomatic controls; M: Male; F: Female; NA: Not available

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT		**Blood-based Diagnostic and Prognostic Models in IBD: The Utility of Serum Calprotectin**	1
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	6 and 7
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2 and 3
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4 and 5
	4	Study objectives and hypotheses	4 and 5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6
Participants	**6**	Eligibility criteria	6
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	6
	8	Where and when potentially eligible participants were identified (setting, location and dates)	6
	9	Whether participants formed a consecutive, random or convenience series	6
Test methods	**10a**	Index test, in sufficient detail to allow replication	6
	10b	Reference standard, in sufficient detail to allow replication	6
	11	Rationale for choosing the reference standard (if alternatives exist)	6
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	6
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	6
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	7
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	7
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	7
	15	How indeterminate index test or reference standard results were handled	7
	16	How missing data on the index test and reference standard were handled	7
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	7
	18	Intended sample size and how it was determined	7
RESULTS			
Participants	**19**	Flow of participants, using a diagram	NA
	20	Baseline demographic and clinical characteristics of participants	8
	21a	Distribution of severity of disease in those with the target condition	8
	21b	Distribution of alternative diagnoses in those without the target condition	8
	22	Time interval and any clinical interventions between index test and reference standard	10
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	8
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	8
	25	Any adverse events from performing the index test or the reference standard	NA
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	13
	27	Implications for practice, including the intended use and clinical role of the index test	13
OTHER INFORMATION			
	28	Registration number and name of registry	6
	29	Where the full study protocol can be accessed	NA
	30	Sources of funding and other support; role of funders	15

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.